L28 ANSWER 6 OF 7 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
ACCESSION NUMBER: 1998:28510016 BIOTECHNO
TITLE: Homologous up-regulation of KDR/Flk-1 receptor expression by vascular endothelial growth factor in vitro
AUTHOR: Shen B.-Q.; Lee D.Y.; Gerber H.-P.; Key B.A.; Ferrara N.; Zioncheck T.F.
CORPORATE SOURCE: T.F. Zioncheck, Dept. of Pharmacokinetics/Metabol., Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, United States.
E-mail: zioncheck.tom@gene.com
SOURCE: Journal of Biological Chemistry, (06 NOV 1998), 273/45
(29979-29985), 62 reference(s)
CODEN: JBCHA3 ISSN: 0021-9258
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28510016 BIOTECHNO
AB We investigated the possibility that vascular endothelial growth factor (VEGF) treatment could regulate KDR/Flk-1 receptor expression in endothelial cells. Bovine adrenal cortex endothelial cells were incubated with 200 pM rhVEGF.sub.1.sub.6.sub.5 for 0-7 days. Western blot analysis showed a 3-5-fold increase in total KDR protein following 4-day VEGF treatment. Scatchard analysis revealed that VEGF induced a 2-3-fold increase in high affinity receptor number (5.0 x 10.sup.4/cell versus 2.4 x 10.sup.4/ cell) without significantly affecting receptor binding affinity (K(d) 76 pM versus 72 pM). Quantitative polymerase chain reaction analysis demonstrated a 3-fold increase in KDR mRNA levels following VEGF exposure. VEGF-induced KDR expression primarily occurred at the transcriptional level as demonstrated by a luciferase **reporter assay** system. Receptor selective mutants with wild-type KDR binding and decreased Flt-1 binding also induced KDR up-regulation; in contrast, mutants with decreased KDR binding and wild-type Flt-1 binding did not, suggesting that KDR receptor signaling mediated the increase in KDR expression. Inhibition of tyrosine kinase, Src tyrosine kinase, protein kinase C, and mitogen-activated protein kinase activities all blocked VEGF- induced KDR up-regulation. Finally, co-incubation of nitric-oxide synthase inhibitors with VEGF had no significant effect on KDR expression, but 100.mu.M sodium nitroprusside, a NO donor, significantly inhibited VEGF-induced KDR up-regulation, indicating that NO negatively regulates KDR expression. In conclusion, our data demonstrate that VEGF binding to the KDR receptor tyrosine kinase results in an increase in KDR receptor gene transcription and protein expression. Thus, KDR up-regulation induced by VEGF may represent an important **positive feedback** mechanism for VEGF action in tumor and ischemia- induced angiogenesis.

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NEWS	5	Mar 20	INPADOC: PRODUCER WARNING ABOUT DATA DELAYS
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FILE 'BIOTECHNO' ENTERED AT 10:43:03 ON 14 MAY 2000

=> s high throughput

L1 3206 HIGH THROUGHPUT

=> s reporter

L2 50715 REPORTER

=> s promoter

L3 188856 PROMOTER

=> s l1 and l2 and l3

L4 22 L1 AND L2 AND L3

=> dup rem l4

PROCESSING COMPLETED FOR L4
L5 10 DUP REM L4 (12 DUPLICATES REMOVED)

=> d ibib abs 1-10

L5 ANSWER 1 OF 10 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2000063730 MEDLINE
DOCUMENT NUMBER: 20063730
TITLE: A yeast screen system for aromatase inhibitors and ligands for androgen receptor: yeast cells transformed with aromatase and androgen receptor.
AUTHOR: Mak P; Cruz F D; Chen S
CORPORATE SOURCE: Wyeth-Ayerst Research, Pearl River, New York, USA.
CONTRACT NUMBER: ES08258 (NIEHS)
SOURCE: ENVIRONMENTAL HEALTH PERSPECTIVES, (1999 Nov) 107 (11) 855-60.
Journal code: EI0. ISSN: 0091-6765.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY WEEK: 20000302

AB Endocrine disruptors are hormone mimics that modify hormonal action in humans and animals. It is thought that some endocrine disruptors modify estrogen and androgen action in humans and animals by suppressing aromatase activity. Aromatase cytochrome P450 is the key enzyme that converts C19 androgens to aromatic C18 estrogenic steroids. We have developed a novel aromatase inhibitor screening method that allows us to identify antiaromatase activity of various environmental chemicals. The screen was developed by coexpressing the human aromatase and the mouse androgen receptor in yeast cells, which carry the androgen-responsive ss-galactosidase **reporter** plasmid. Functional expression of aromatase in yeast has been demonstrated using the [3H]-water release assay with intact cells as well as with yeast microsomes. The aromatase activity could be blocked by known aromatase inhibitors such as aminoglutethimide (AG). Yeast-produced androgen receptors were able to transactivate a yeast basal **promoter** linked to an androgen-responsive element in response to androgens. The resultant triple yeast transformant responded to the treatment of testosterone, androstenedione, or 5 alpha-dihydrotestosterone (5 alpha-DHT). In the absence of the aromatase inhibitor AG, transcriptional activation was observed only for the nonaromatizable androgen 5 alpha-DHT. However, the two aromatizable androgens (testosterone and androstenedione) induced the **reporter** activity in the presence of AG. Using this yeast-based assay, we confirmed that two flavones, chrysin and alpha-naphtholflavone, are inhibitors of aromatase. Thus, this yeast system allows us to develop

a **high-throughput** screening method, without using radioactive substrate, to identify aromatase inhibitors as well as new ligands (nonaromatizable androgen mimics) for the androgen receptors. In addition, this screening method also allows us to distinguish nonandrogenic aromatase inhibitors from inhibitors with androgenic activity. This yeast screening method will be useful to screen environmental chemicals for their antiaromatase activity and for their interaction with androgen receptor.

L5 ANSWER 2 OF 10 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000045093 MEDLINE
DOCUMENT NUMBER: 20045093
TITLE: Expression of cre recombinase as a **reporter** of signal transduction in mammalian cells.
AUTHOR: Mattheakis L C; Olivan S E; Dias J M; Northrop J P
CORPORATE SOURCE: Affymax Research Institute, Palo Alto, CA 94304, USA.. larry_mattheakis@affymax.com
SOURCE: CHEMISTRY AND BIOLOGY, (1999 Nov) 6 (11) 835-44. Journal code: CNA. ISSN: 1074-5521.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY WEEK: 20000401
AB BACKGROUND: Cell-based **reporter** assays, which rely on a **reporter** gene under the control of a regulated **promoter**, are widely used to screen chemical libraries for novel receptor ligands. Here, we describe a **reporter** system that is based on ligand-induced DNA recombination to express the **reporter** gene. This system converts a transient activation of a signal transduction pathway into an amplified, constitutive and heritable expression of the **reporter** gene. RESULTS: We constructed gene fusions of Cre recombinase and mammalian promoters regulated by calcium, nuclear receptors or cyclic AMP. **Reporter** systems, comprising a Cre gene fusion and a loxP/**reporter** gene, were used to study the kinetics and dose responses to compounds that activate or inhibit the corresponding signal transduction pathway. We compared these reporters with conventional **reporter** systems in which the **reporter** gene is under the direct control of the responsive **promoter**. **Reporter** gene expression of the Cre reporters was greater than that of conventional reporters and could be measured more than a week after adding the stimulus. For all pathways studied here, the dose responses of the Cre reporters are nearly identical to those of conventional **reporter** systems. CONCLUSIONS: We have shown that Cre recombinase can be regulated by a variety of signal transduction pathways. It should therefore be possible to use receptor ligands to induce phenotypic conversion of mammalian cells for use in a variety of applications. One such application is **high-throughput** screening, and we developed loxP/luciferase **reporter** genes that provide an amplified and sustained luminescent response.

L5 ANSWER 3 OF 10 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 1999352424 MEDLINE
DOCUMENT NUMBER: 99352424
TITLE: Rapid fluorescence-based **reporter**-gene assays to evaluate the cytotoxicity and antitumor drug potential of platinum complexes.
AUTHOR: Sandman K E; Marla S S; Zlokarnik G; Lippard S J
CORPORATE SOURCE: Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
CONTRACT NUMBER: CA34992 (NCI)
CA09112 (NCI)
SOURCE: CHEMISTRY AND BIOLOGY, (1999 Aug) 6 (8) 541-51. Journal code: CNA. ISSN: 1074-5521.

PUB. COUNTRY: [redacted]NGLAND: United Kingdom
[redacted]ournal; Article; (JOURNAL ARTIC[redacted]
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY WEEK: 19991204
AB BACKGROUND: The need for new platinum antitumor drugs is underscored by the usefulness of cisplatin and carboplatin in chemotherapy and the resistance of many tumors to these compounds. Combinatorial chemistry could aid in the search for cisplatin analogs if fast, **high-throughput** assays were available. Our goal was to develop rapid cell-based assays suitable for **high-throughput** screening that accurately predict the cytotoxicity of platinum complexes. We examined the effects of platinum complexes and other agents on **reporter**-gene expression in cancer cells. RESULTS: HeLa Tet-On cells with inducible enhanced green fluorescent protein (EGFP) were prepared. Cisplatin and other cis-disubstituted platinum complexes inhibited EGFP expression, with a strong positive correlation between EGFP inhibition and cytotoxicity. By contrast, trans-[Pt(NH(3))(2)Cl(2)], other trans-platinum complexes, methyl methanesulfonate or heat shock stimulated EGFP expression. Northern and nuclear run-on analyses revealed that the changes in EGFP expression were at the level of transcription. In another **reporter**-gene assay in Jurkat cells, cisplatin, but not trans-[Pt(NH(3))(2)Cl(2)] or K(2)[PtCl(4)], inhibited beta-lactamase expression, as measured by hydrolysis of the fluorescent substrate CCF2. CONCLUSIONS: The EGFP results indicate that cytotoxic stress enhances transcription from the inducible **promoter**, whereas compounds able to form the 1,2-intrastrand platinum-DNA cross-links repress transcription. Both fluorescence-based **reporter**-gene assays afford promising new approaches to platinum anticancer drug discovery.

L5 ANSWER 4 OF 10 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2000013177 MEDLINE
DOCUMENT NUMBER: 20013177
TITLE: New molecular bioassays for the estimation of the teratogenic potency of valproic acid derivatives in vitro: activation of the peroxisomal proliferator-activated receptor (PPARdelta).
AUTHOR: Lampen A; Siehler S; Ellerbeck U; Gottlicher M; Nau H
CORPORATE SOURCE: Zentrumsabteilung fur Lebensmitteltoxikologie, Tierarztliche Hochschule Hannover, Hannover, Germany.
SOURCE: TOXICOLOGY AND APPLIED PHARMACOLOGY, (1999 Nov 1) 160 (3) 238-49.
Journal code: VWO. ISSN: 0041-008X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 200002
ENTRY WEEK: 20000204
AB Therapy with the antiepileptic drug valproic acid (2-propylpentanoic acid, VPA) during early pregnancy can cause teratogenic effects (neural tube defects) in humans and in mice. VPA and a teratogenic derivative specifically induce differentiation of F9 teratocarcinoma cells and activate PPARdelta. We have now studied structure-activity relationships of 11 VPA-related compounds by quantitatively comparing their teratogenic potency with their effects in the two novel in vitro systems. Based on the induction of a Rous sarcoma virus (RSV) **promoter**-driven **reporter** gene, which is associated with the differentiation of F9 cells, a system suitable for **high-throughput** and quantitative screening was established. Structure-activity investigations showed that only teratogenic derivatives of VPA induced the response in F9 cells as well as activated the PPARdelta-dependent **reporter**

system in Chinese hamster ovary (CHO) cells. Increases in the length of the side chain in the VPA-related 2-alkyl-pentanoic acid generate more potent inducers in the cell-culture-based assays, which also show higher teratogenicity and embryonic lethality rates. Activation of PPARdelta correlated well with the effects in the F9 cell assay and with teratogenic potency in vivo ($p < 0.007$). Evaluation of the effects of the presented set of compounds allows the conclusion that the in vitro systems faithfully reflect teratogenicity of VPA-related compounds. Whether the activation of PPARdelta is causally related to the disruption of proper embryonic development or whether it reflects other yet unknown VPA-induced events remains to be established. Copyright 1999 Academic Press.

L5 ANSWER 5 OF 10 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 2000053849 MEDLINE
DOCUMENT NUMBER: 20053849
TITLE: A cellular **reporter** assay to monitor insulin receptor kinase activity based on STAT 5-dependent luciferase gene expression.
AUTHOR: Storz P; Doppler H; Horn-Muller J; Groner B; Pfizenmaier K; Muller G
CORPORATE SOURCE: Institute of Cell Biology and Immunology, University of Stuttgart, Stuttgart, D-70569, Germany.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1999 Dec 1) 276 (1) 97-104. Journal code: 4NK. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY WEEK: 20000403
AB A highly sensitive method for determination of insulin receptor (IR) kinase activity in whole cells, which is based on a STAT5 (signal transducer and activator of transcription 5)-dependent **reporter** gene assay, has been developed. We show in Rat1 fibroblasts stably overexpressing the human IR (Rat1-HIR-c15) an insulin-dependent direct association and phosphorylation of STAT5b by IR kinase. Rat1-HIR cells transfected with a luciferase gene **reporter** construct under control of a STAT5-inducible **promoter** showed insulin-mediated induction of STAT5-dependent luciferase activity, with peak activities around 8 h of insulin treatment over a wide dose range. Transient STAT5b but not STAT5a cotransfection significantly enhanced **reporter** gene activity, yielding up to a fivefold induction. Addition of the IR kinase inhibitor tyrphostin AG1024 down-regulated luciferase induction in a dose-dependent manner. This is the first assay allowing determination of IR kinase activity in intact cells in a 24-well culture and a microtiter format. Kinetics of this cellular response, sensitivity range, and signal amplitude make it well suited for automation and offer the potential for establishing **high-throughput** screening systems for both insulin mimetic substances and IR kinase antagonists in a simple nonradioactive assay. Copyright 1999 Academic Press.

L5 ANSWER 6 OF 10 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 2000011146 MEDLINE
DOCUMENT NUMBER: 20011146
TITLE: Measurement of responses from Gi-, Gs-, or Gq-coupled receptors by a multiple response element/cAMP response element-directed **reporter** assay.
AUTHOR: Fitzgerald L R; Mannan I J; Dytko G M; Wu H L; Nambi P
CORPORATE SOURCE: Department of Renal Pharmacology, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, Pennsylvania 19406, USA.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1999 Nov 1) 275 (1) 54-61. Journal code: 4NK. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY WEEK: 20000204
AB We have established a rapid, sensitive, **high-throughput** assay that requires one assay condition to detect agonist effects from Gi-, Gs-, and Gq-coupled receptors. We utilized a vector containing a **promoter** with three multiple response elements, the vasoactive intestinal peptide **promoter** and a cAMP response element controlling the transcription of the luciferase gene. An adrenergic agonist, para-aminoclonidine, inhibited forskolin-stimulated luciferase expression when cells were cotransfected with the Gi-coupled alpha(2)-C adrenergic receptor and the MRE/CRE **reporter** vector. Further, we demonstrate that gastrin-releasing peptide, which activates a Gq-coupled GRP receptor, isoproterenol, which activates a Gs-coupled beta-adrenergic receptor, calcium ionophores, and phorbol 12-myristate 13-acetate, a stimulator of protein kinase C, can mediate increases in luciferase expression in the presence of forskolin but not in its absence. The effect at Gi-coupled receptor activation correlates with the phosphorylation of the CRE binding protein (CREB); however, the mechanisms mediating the responses to Gq- and Gs-coupled receptors are more complex. We demonstrate that this assay is useful for pharmacological analysis of both agonists and antagonists and has the potential to associate orphan G-protein-coupled receptors with their corresponding ligands. Copyright 1999 Academic Press.

L5 ANSWER 7 OF 10 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
ACCESSION NUMBER: 1998:28297219 BIOTECHNO
TITLE: Use of transgenic animals in understanding molecular mechanisms of toxicity
AUTHOR: Wolf C.R.; Henderson C.J.
CORPORATE SOURCE: C.J. Henderson, Imperial Cancer Research Fund, Biomedical Research Centre, Ninewells Hospital Medical School, Dundee DD1 9SY, United Kingdom.
E-mail: m.rooney@icrf.icnet.uk
SOURCE: Journal of Pharmacy and Pharmacology, (1998), 50/6 (567-574), 48 reference(s)
CODEN: JPPMAB ISSN: 0022-3573
DOCUMENT TYPE: Journal; Conference Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28297219 BIOTECHNO
AB Understanding molecular mechanisms of chemical toxicity and the potential risks of drugs to man is a pivotal part of the drug development process. With the dramatic increase in the number of new chemical entities arising from **high throughput** screening, there is an urgent need to develop systems for the rapid evaluation of potential drugs so that those agents which are most likely to be free of adverse effects can be identified at the earliest possible stage in drug development. The complex mechanisms of action of chemical toxins has made it extremely difficult to evaluate the precise toxic mechanism and also the relative role of specific genes in either potentiating or ameliorating the toxic effect. This problem can be addressed by the application of genetic strategies. Such strategies can exploit strain differences in susceptibility to specific toxic agents or, with the rapidly developing technologies, can exploit the use of transgenic animals where specific genes can be manipulated and subsequent effects on chemical toxicity evaluated. Transgenic animals can be exploited in a variety of ways to understand mechanisms of chemical toxicity. For example, a human gene encoding a drug metabolizing enzyme can be directly introduced and the effects on toxic response evaluated. Alternatively, specific genes can be

deleted from the mouse genome and the consequences on toxicological response determined. Many toxic chemical agents modulate patterns of gene expression within target cells. This can be used to screen for responses to different types of toxic insult. In such experiments, the promotor of a stress-regulated gene can be ligated to a suitable **reporter** gene, such as lacZ, or green fluorescent protein, and inserted into the genome of an appropriate test species. On administration of a chemical agent, cells which are sensitive to the toxic effects of that chemical will express the **reporter**, which can then be identified using an appropriate assay system. This latter strategy provides the potential for screening a large number of compounds rapidly for their potential toxic effects and also provides information on tissue and cellular specificity. Experiments using transgenic animals can be complex, and care must be taken to ensure that the results are not affected by background activities within the species being used. For example, the introduction of a specific human cytochrome P450 gene may have no effect on the metabolic disposition of a drug or toxin because of the background activity within the mouse. As the toxicity of a chemical agent is determined by a wide range of different factors including drug uptake, metabolism, detoxification and repair, differences between man and the species being used could potentially generate a toxic response in the animal model whereas no toxicity may be observed in man. In spite of these confounding factors, the application of transgenic animals to toxicological issues has enormous potential for speeding up the drug discovery process and will undoubtedly become part of this process in the future.

L5 ANSWER 8 OF 10 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1998:428112 BIOSIS
DOCUMENT NUMBER: PREV199800428112
TITLE: Development of a **high-throughput** in vitro bioassay to assess potencies of progestagenic compounds using Chinese hamster ovary cells stably transfected with the human progesterone receptor and a luciferase **reporter** system.
AUTHOR(S): Schoonen, Willem G. E. J.; De Ries, Rob J. H.; Joosten, Jan W. H.; Mathijssen-Mommers, Gerrie J. W.; Kloosterboer, Helenius J.
CORPORATE SOURCE: Dep. Endocrinol., N.V. Organon, Molenstr. 110, P.O. Box 20, 5340 BH Oss Netherlands
SOURCE: Analytical Biochemistry, (Aug. 1, 1998) Vol. 261, No. 2, pp. 222-224.
ISSN: 0003-2697.
DOCUMENT TYPE: Article
LANGUAGE: English

L5 ANSWER 9 OF 10 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 1998197099 MEDLINE
DOCUMENT NUMBER: 98197099
TITLE: Genetic recombination as a **reporter** for screening steroid receptor agonists and antagonists.
AUTHOR: Dias J M; Go N F; Hart C P; Mattheakis L C
CORPORATE SOURCE: Affymax Research Institute, 4001 Miranda Avenue, Palo Alto, California 94304, USA.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1998 Apr 10) 258 (1) 96-102.
Journal code: 4NK. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY WEEK: 19980701

AB **Reporter** cell lines are often used for **high throughput** screening of chemical libraries to identify new receptor ligands. Here we show how Cre recombinase can be used in mammalian cells to screen for steroid receptor ligands. A translational fusion of Cre recombinase and the ligand binding domain of the human glucocorticoid receptor was transfected into mammalian cells with a loxP/luciferase **reporter** gene. The recombinase function of the fusion is dependent on ligand binding to the receptor, and Cre-mediated recombination results in constitutive expression of luciferase from the **reporter** gene. A stable transfected clone was isolated and used to characterize the kinetics, ligand specificity, and dose response to various receptor ligands. The Cre fusion system, unlike a transcriptional **reporter** using the mouse mammary tumor virus **promoter**, can detect binding of the receptor antagonist RU486. We also studied the Cre **reporter** in a sensitive, miniaturized, assay format using an 864-well plate and show that as few as 560 cells per assay well was sufficient to measure a dose response to ligand. Copyright 1998 Academic Press.

L5 ANSWER 10 OF 10 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 95000561 MEDLINE
DOCUMENT NUMBER: 95000561
TITLE: A simple and sensitive **high-throughput** assay for steroid agonists and antagonists.
AUTHOR: White J H; McCuaig K A; Mader S
CORPORATE SOURCE: Department of Physiology, McGill University, Montreal, Que., Canada.
SOURCE: BIO/TECHNOLOGY, (1994 Oct) 12 (10) 1003-7.
Journal code: AL1. ISSN: 0733-222X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: B
ENTRY MONTH: 199501
AB We have developed a simple and highly sensitive tissue culture-based assay for the biological activity of steroids and synthetic steroidal compounds. A DNA cassette, containing a synthetic steroid-inducible **promoter** controlling the expression of a bacterial chloramphenicol acetyltransferase gene (GRE5-CAT), was inserted into an Epstein-Barr virus (EBV) episomal vector which replicates autonomously in primate and human cells. We then used this **promoter/reporter** system to generate two stably transfected human cell lines. In the cervical carcinoma cell line HeLa, which expresses high levels of glucocorticoid receptor, the GRE5 **promoter** is inducible over 100-fold by the synthetic glucocorticoid dexamethasone. In the breast carcinoma cell line T47D, which expresses progesterone and androgen receptors, the GRE5 **promoter** is inducible over 100-fold by either progesterone or dihydrotestosterone. In both cell lines basal expression of CAT activity is strictly dependent on the presence of steroid, so that very low levels of induction can be detected. Thus, the cell lines can be used to test for low levels of agonist activity in steroid antagonists. These cell lines can be used to screen compounds for steroid agonist or antagonist activity by testing extracts of cells grown in microtiter wells directly using a colorimetric CAT assay. This system should provide a sensitive and efficient method for screening and analysis of the activity of large numbers of natural or synthetic steroid agonists or antagonists.

=> d his

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FILE 'MEDLINE, BIOSIS, BIOTECHNO' ENTERED AT 10:43:03 ON 14 MAY 2000

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L1          3206 S HIGH THROUGHPUT
L2         50715 S REPORTER
L3        188856 S PROMOTER
L4            22 S L1 AND L2 AND L3
L5            10 DUP REM L4 (12 DUPLICATES REMOVED)

=> l1 and l2

L1 IS NOT A RECOGNIZED COMMAND
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"HELP COMMANDS" at an arrow prompt (=>).

=> s l1 and l2

L6          117 L1 AND L2

=> s l6 and amplif

L7            0 L6 AND AMPLIF

=> s amplif? or cooperative or synergis?

L8       316071 AMPLIF? OR COOPERATIVE OR SYNERGIS?

=> s l8 and l6

L9            8 L8 AND L6

=> dup rem l9

PROCESSING COMPLETED FOR L9
L10           4 DUP REM L9 (4 DUPLICATES REMOVED)

=> d ibib abs 1-4


L10  ANSWER 1 OF 4  MEDLINE                                  DUPLICATE 1
ACCESSION NUMBER:   2000145745      MEDLINE
DOCUMENT NUMBER:    20145745
TITLE:              Multiplexed single nucleotide polymorphism genotyping by
                    oligonucleotide ligation and flow cytometry.
AUTHOR:             Iannone M A; Taylor J D; Chen J; Li M S; Rivers P;
                    Slentz-Kesler K A; Weiner M P
CORPORATE SOURCE:   Department of Molecular Sciences, Glaxo Wellcome Research
                    Laboratories, Research Triangle Park, NC 27709-3398, USA..
                    mai49583@glaxowellcome.com
SOURCE:             CYTOMETRY, (2000 Feb 1) 39 (2) 131-40.
                    Journal code: D92. ISSN: 0196-4763.
PUB. COUNTRY:       United States
                    Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200005
ENTRY WEEK:         20000502
AB   BACKGROUND: We have developed a rapid, high throughput
     method for single nucleotide polymorphism (SNP) genotyping that employs
an
     oligonucleotide ligation assay (OLA) and flow cytometric analysis of
     fluorescent microspheres. METHODS: A fluoresceinated oligonucleotide
     reporter sequence is added to a "capture" probe by OLA. Capture
     probes are designed to hybridize both to genomic "targets"
     amplified by polymerase chain reaction and to a separate
     complementary DNA sequence that has been coupled to a microsphere. These
     sequences on the capture probes are called "ZipCodes". The OLA-modified
     capture probes are hybridized to ZipCode complement-coupled microspheres.
     The use of microspheres with different ratios of red and orange
     fluorescence makes a multiplexed format possible where many SNPs may be
     analyzed in a single tube. Flow cytometric analysis of the microspheres
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simultaneously identifies both the microsphere type and the fluorescent green signal associated with the SNP genotype. RESULTS: Application of this methodology is demonstrated by the multiplexed genotyping of seven CEPH DNA samples for nine SNP markers located near the ApoE locus on chromosome 19. The microsphere-based SNP analysis agreed with genotyping by sequencing in all cases. CONCLUSIONS: Multiplexed SNP genotyping by OLA with flow cytometric analysis of fluorescent microspheres is an accurate and rapid method for the analysis of SNPs. Copyright 2000 Wiley-Liss, Inc.

L10 ANSWER 2 OF 4 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000045093 MEDLINE
DOCUMENT NUMBER: 20045093
TITLE: Expression of cre recombinase as a **reporter** of signal transduction in mammalian cells.
AUTHOR: Mattheakis L C; Olivan S E; Dias J M; Northrop J P
CORPORATE SOURCE: Affymax Research Institute, Palo Alto, CA 94304, USA.. larry_mattheakis@affymax.com
SOURCE: CHEMISTRY AND BIOLOGY, (1999 Nov) 6 (11) 835-44. Journal code: CNA. ISSN: 1074-5521.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY WEEK: 20000401
AB BACKGROUND: Cell-based **reporter** assays, which rely on a **reporter** gene under the control of a regulated promoter, are widely used to screen chemical libraries for novel receptor ligands. Here, we describe a **reporter** system that is based on ligand-induced DNA recombination to express the **reporter** gene. This system converts a transient activation of a signal transduction pathway into an **amplified**, constitutive and heritable expression of the **reporter** gene. RESULTS: We constructed gene fusions of Cre recombinase and mammalian promoters regulated by calcium, nuclear receptors or cyclic AMP. **Reporter** systems, comprising a Cre gene fusion and a loxP/**reporter** gene, were used to study the kinetics and dose responses to compounds that activate or inhibit the corresponding signal transduction pathway. We compared these reporters with conventional **reporter** systems in which the **reporter** gene is under the direct control of the responsive promoter. **Reporter** gene expression of the Cre reporters was greater than that of conventional reporters and could be measured more than a week after adding the stimulus. For all pathways studied here, the dose responses of the Cre reporters are nearly identical to those of conventional **reporter** systems. CONCLUSIONS: We have shown that Cre recombinase can be regulated by a variety of signal transduction pathways. It should therefore be possible to use receptor ligands to induce phenotypic conversion of mammalian cells for use in a variety of applications. One such application is **high-throughput** screening, and we developed loxP/luciferase **reporter** genes that provide an **amplified** and sustained luminescent response.

L10 ANSWER 3 OF 4 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
ACCESSION NUMBER: 1998:28163149 BIOTECHNO
TITLE: Genetic recombination as a **reporter** for screening steroid receptor agonists and antagonists
AUTHOR: Dias J.M.; Ning Fei Go; Hart C.P.; Mattheakis L.C.
CORPORATE SOURCE: L.C. Mattheakis, Affymax Research Institute, 4001 Miranda Avenue, Palo Alto, CA 94304, United States.
SOURCE: Analytical Biochemistry, (10 APR 1998), 258/1 (96-102), 24 reference(s)
CODEN: ANBCA2 ISSN: 0003-2697
DOCUMENT TYPE: Journal; Article

COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28163149 BIOTECHNO
AB **Reporter** cell lines are often used for **high throughput** screening of chemical libraries to identify new receptor ligands. Here we show how Cre recombinase can be used in mammalian cells to screen for steroid receptor ligands. A translational fusion of Cre recombinase and the ligand binding domain of the human glucocorticoid receptor was transfected into mammalian cells with a loxP/luciferase **reporter** gene. The recombinase function of the fusion is dependent on ligand binding to the receptor, and Cre-mediated recombination results in constitutive expression of luciferase from the **reporter** gene. A stable transfected clone was isolated and used to characterize the kinetics, ligand specificity, and dose response to various receptor ligands. The Cre fusion system, unlike a transcriptional **reporter** using the mouse mammary tumor virus promoter, can detect binding of the receptor antagonist RU486. We also studied the Cre **reporter** in a sensitive, miniaturized, assay format using an 864-well plate and show that as few as 560 cells per assay well was sufficient to measure a dose response to ligand.

L10 ANSWER 4 OF 4 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 97064969 MEDLINE
DOCUMENT NUMBER: 97064969
TITLE: A novel method for real time quantitative RT-PCR.
AUTHOR: Gibson U E; Heid C A; Williams P M
CORPORATE SOURCE: Genentech, Inc., South San Francisco, California 94080-4990, USA.
SOURCE: GENOME RESEARCH, (1996 Oct) 6 (10) 995-1001.
Journal code: CES. ISSN: 1088-9051.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY WEEK: 19970403
AB A novel approach to quantitative reverse transcriptase polymerase chain reaction (QC RT-PCR) using real time detection and the 5' nuclease assay has been developed. Cystic fibrosis transmembrane transductance regulator (CFTR) target mRNA is reverse transcribed, **amplified**, detected, and quantitated in real time. A fluorogenic probe was designed to detect the CFTR amplicon. Relative increase in 6-carboxy-fluorescein **reporter** fluorescent emission is monitored during PCR **amplification** using an analytical thermal cycler. An internal control template containing the same primer sequences as the CFTR amplicon, but a different internal sequence, has been designed as a control. An internal control probe with a **reporter** fluorescent dye tetrachloro-6-carboxy-fluorescein was designed to hybridize to the internal control amplicon. The internal control template is placed in each reaction tube and is used for quantitative analysis of the CFTR mRNA. This method provides a convenient and **high-throughput** format for QC RT-PCR.

=> d his

(FILE 'HOME' ENTERED AT 10:42:07 ON 14 MAY 2000)

FILE 'MEDLINE, BIOSIS, BIOTECHNO' ENTERED AT 10:43:03 ON 14 MAY 2000
L1 3206 S HIGH THROUGHPUT
L2 50715 S REPORTER
L3 188856 S PROMOTER
L4 22 S L1 AND L2 AND L3
L5 10 DUP REM L4 (12 DUPLICATES REMOVED)

```
L6          117 S L1 AND L2
L7            0 S L6 AND AMPLIF
L8       316071 S AMPLIF? OR COOPERATIVE OR SYNERGIS?
L9            8 S L8 AND L6
L10           4 DUP REM L9 (4 DUPLICATES REMOVED)

=> s l6 not l9

L11         109 L6 NOT L9

=> s l6 and (all or none)

L12          11 L6 AND (ALL OR NONE)

=> s l6 and map

L13           3 L6 AND MAP

=> s l6 and map?

L14           5 L6 AND MAP?

=> dup rem l14

PROCESSING COMPLETED FOR L14
L15           4 DUP REM L14 (1 DUPLICATE REMOVED)

=> d ibib abs 1-4


L15  ANSWER 1 OF 4  MEDLINE
ACCESSION NUMBER:   1999117537     MEDLINE
DOCUMENT NUMBER:    99117537
TITLE:              A high throughput system for the
                    evaluation of protein kinase C inhibitors based on Elk1
                    transcriptional activation in human astrocytoma cells.
AUTHOR:             Sharif T R; Sharif M
CORPORATE SOURCE:   Department of Molecular Pharmacology, St. Jude Children's
                    Research Hospital, Memphis, TN 38105, USA.
CONTRACT NUMBER:    CA71756 (NCI)
                    CA21765 (NCI)
SOURCE:             INTERNATIONAL JOURNAL OF ONCOLOGY, (1999 Feb) 14 (2)
                    327-35.
                    Journal code: CX5. ISSN: 1019-6439.
PUB. COUNTRY:       Greece
                    Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        199904
ENTRY WEEK:         19990403
AB   Protein kinase C (PKC) designates a family of kinases that regulate many
     essential functions including cell growth and differentiation. The tight
     regulation of PKC activity is crucial for maintaining normal cellular
     proliferation and excessive activity leads to abnormal or uncontrolled
     cell growth. Recent reports indicate that malignant glioma cell lines
     express 100 to 1000-fold higher PKC activity when compared to
     non-neoplastic astrocytes. This high activity correlates well with the
     proliferation of tumor cells in vitro. We recently reported on the
     anti-proliferative properties of selective PKC inhibitors on the growth
of
     U-373MG human astrocytoma cell line, and their ability to block
     mitogen-activated protein (MAP) kinase pathway activated by
     substance P (SP) neuropeptide receptor signaling via a PKC-dependent
     mechanism. Therefore, inhibiting PKC activity by selective PKC inhibitors
     may present a promising approach for improving astroglial brain tumor
     therapy. For this purpose, we constructed a high
     throughput model cell system to evaluate the efficacy of PKC
     inhibitors. This system is based on the measurement of light production
in
```

U-373MG cells stably transfected with the luciferase **reporter** gene whose expression depends on the transcriptional activation of GAL4-Elk1 fusion protein by enzyme components of the **MAP** kinase pathway and the upstream activation of PKC (PKC activation-->**MAP** kinases-->GAL4-Elk1 phosphorylation-->luciferase expression-->luciferase activity). In brief, we have demonstrated that the PKC activator 12-O-tetradecanoyl phorbol 13-acetate (TPA)-induced luciferase activity in this cell system is mediated via the **MAP** kinase pathway and can be blocked in the presence of MEK1 selective inhibitors (PD 098059 or U0126). We also demonstrated that TPA-induced luciferase activity in U-373MG stable clones can be blocked by PKC inhibitors (CGP 41251, Go 6976, and GF 109203X) in a concentration dependent manner. In contrast, epidermal growth factor (EGF)-induced luciferase activity, which is independent of PKC activation (Ras-->Raf-1-->MEK1-->**MAP** kinases-->GAL4-Elk1 phosphorylation-->luciferase expression-->luciferase activity) can only be blocked using a selective EGF receptor inhibitor (AG 1478). In conclusion, we have constructed a model cell system for the **high throughput** screening and identification of PKC inhibitors potentially active against astrocytoma cells in culture.

L15 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1999:120716 BIOSIS
DOCUMENT NUMBER: PREV199900120716
TITLE: A **high throughput** system for the evaluation of protein kinase C inhibitors based on Elk1 transcriptional activation in human astrocytoma cells.
AUTHOR(S): Sharif, Taraneh R.; Sharif, Mohammed (1)
CORPORATE SOURCE: (1) Dep. Mol. Pharmacol., St. Jude Child. Res. Hosp., 332 North Lauderdale, Memphis, TN 38105 USA
SOURCE: International Journal of Oncology, (Feb., 1999) Vol. 14, No. 2, pp. 326-335.
ISSN: 1019-6439.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Protein kinase C (PKC) designates a family of kinases that regulate many essential functions including cell growth and differentiation. The tight regulation of PKC activity is crucial for maintaining normal cellular proliferation and excessive activity leads to abnormal or uncontrolled cell growth. Recent reports indicate that malignant glioma cell lines express 100 to 1000-fold higher PKC activity when compared to non-neoplastic astrocytes. This high activity correlates well with the proliferation of tumor cells in vitro. We recently reported on the anti-proliferative properties of selective PKC inhibitors on the growth of U-373MG human astrocytoma cell line, and their ability to block mitogen-activated protein (**MAP**) kinase pathway activated by substance P (SP) neuropeptide receptor signaling via a PKC-dependent mechanism. Therefore, inhibiting PKC activity by selective PKC inhibitors may present a promising approach for improving astroglial brain tumor therapy. For this purpose, we constructed a **high throughput** model cell system to evaluate the efficacy of PKC inhibitors. This system is based on the measurement of light production in U-373MG cells stably transfected with the luciferase **reporter** gene whose expression depends on the transcriptional activation of GAL4-Elk1 fusion protein by enzyme components of the **MAP** kinase pathway and the upstream activation of PKC (PKC activation-MA kinases-GAL4-Elk1 phosphorylation fwdarw luciferase expression-luciferase activity). In brief, we have demonstrated that the PKC activator 12-O-tetradecanoyl phorbol 13-acetate (TPA)-induced luciferase activity in this cell system is mediated via the **MAP** kinase pathway and can be blocked in the presence of MEK1 selective inhibitors (PD 098059 or U0126). We also demonstrated that TPA-induced luciferase activity in U-373MG stable clones can be blocked by PKC inhibitors (CGP 41251, Go 6976, and GF 109203X) in a concentration dependent manner. In contrast, epidermal growth factor (EGF)-induced luciferase activity, which is

independent of PKC activation (Ras fwdarw Raf-1 fwdarw MEK1 fwdarw **MAP** kinases-GAL4-Elk1 phosphorylation-luciferase expression-luciferase activity), can only be blocked using a selective EGF receptor inhibitor (AG 1478). In conclusion, we have constructed a model cell system for the **high throughput** screening and identification of PKC inhibitors potentially active against astrocytoma cells in culture.

L15 ANSWER 3 OF 4 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1998:349893 BIOSIS
DOCUMENT NUMBER: PREV199800349893
TITLE: Ro 09-2210 exhibits potent anti-proliferative effects on activated T cells by selectively blocking MKK activity.
AUTHOR(S): Williams, D. H.; Wilkinson, S. E.; Purton, T.; Lamont, A.; Flotow, H.; Murray, E. J. (1)
CORPORATE SOURCE: (1) Roche Res. Centre, P.O. Box 8, Welwyn Garden City, Herts AL7 3AU UK
SOURCE: Biochemistry, (June 30, 1998) Vol. 37, No. 26, pp. 9579-9585.
ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
AB By using **high throughput** screening of microbial broths, we have identified a compound, designated Ro 09-2210, which is able to block anti-CD3 induced peripheral blood T cell activation with an IC50 = 40 nM. Ro 09-2210 was also able to block antigen-induced IL-2 secretion with an IC50 = 30 nM, but was considerably less potent at blocking Ca2+ flux stimulated by anti-CD3 treatment. To determine the mechanism of action of Ro 09-2210, we set up a transient expression system in Jurkat T cells using a variety of **reporter** gene constructs and showed effective inhibition of phorbol ester/ionomycin-induced NF-AT activation and anti-CD3 induced NF-AT with IC50 = 7.7 and 10 nM, respectively. Ro 09-2210 was also able to inhibit phorbol ester/ionomycin-induced activation of AP1 with IC50 = < 10 nM. We further showed that Ro 09-2210 was unable to inhibit c-jun induced expression of AP1-dependent **reporter** constructs (IC50 > 500 nM), but was able to potently inhibit ras-induced AP1 activation (IC50 = 20 nM). This suggested that Ro 09-2210 was inhibiting an activator of AP-1 which was upstream of c-jun and downstream of ras signaling. To investigate further, we then purified a number of different kinases, including PKC, PhK, ZAP-70, ERK, and MEK 1 (a MKK), and showed that Ro 09-2210 was a selective inhibitor of MEK1 in vitro (IC50 = 59 nM).

L15 ANSWER 4 OF 4 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1998158834 MEDLINE
DOCUMENT NUMBER: 98158834
TITLE: RNase L dimerization in a mammalian two-hybrid system in response to 2',5'-oligoadenylates.
AUTHOR: Naik S; Paranjape J M; Silverman R H
CORPORATE SOURCE: Department of Cancer Biology, NN1-06, The Lerner Research Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, USA.
CONTRACT NUMBER: CA 44059 (NCI)
SOURCE: NUCLEIC ACIDS RESEARCH, (1998 Mar 15) 26 (6) 1522-7.
Journal code: O8L. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199806
ENTRY WEEK: 19980604
AB RNase L, a key enzyme in the anti-viral activity of interferons, requires activation by 2',5'-linked oligoadenylates (2-5A) to cleave viral and cellular single-stranded RNA. Here we demonstrate that 2-5A causes

formation of stable dimers of RNase L in intact human cells as measured with a mammalian two-hybrid system. Hybrid proteins consisting of the GAL4 DNA binding domain fused to RNase L and the VP16 transactivation domain fused to RNase L were able to associate and drive transcription of a **reporter** gene, but only after cells were transfected with 2-5A. Several functional forms of 2-5A, such as p3A2'p5'A2'p5'A, were capable of activating transcription in human HeLa cells. In contrast, p3A2'p5'A, which can neither activate nor dimerize RNase L, did not induce gene expression. Evidence for the involvement of the C-terminal region of RNase L in dimerization was obtained by expressing truncated forms of RNase L. These findings describe a convenient, **high-throughput** screening method for RNase L activators which could lead to the discovery of novel anti-viral and anti-cancer agents.

=> d his

(FILE 'HOME' ENTERED AT 10:42:07 ON 14 MAY 2000)

FILE 'MEDLINE, BIOSIS, BIOTECHNO' ENTERED AT 10:43:03 ON 14 MAY 2000

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L1          3206 S HIGH THROUGHPUT
L2         50715 S REPORTER
L3        188856 S PROMOTER
L4            22 S L1 AND L2 AND L3
L5            10 DUP REM L4 (12 DUPLICATES REMOVED)
L6           117 S L1 AND L2
L7             0 S L6 AND AMPLIF
L8        316071 S AMPLIF? OR COOPERATIVE OR SYNERGIS?
L9             8 S L8 AND L6
L10            4 DUP REM L9 (4 DUPLICATES REMOVED)
L11          109 S L6 NOT L9
L12           11 S L6 AND (ALL OR NONE)
L13            3 S L6 AND MAP
L14            5 S L6 AND MAP?
L15            4 DUP REM L14 (1 DUPLICATE REMOVED)
```

=> s autopositive

L16 5 AUTOPOSITIVE

=> feedforward

FEEDFORWARD IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s feedforward

L17 1162 FEEDFORWARD

=> s positive feedback

L18 4220 POSITIVE FEEDBACK

=> s l16 or l17 or l18

L19 5377 L16 OR L17 OR L18

=> s l6 and l19

L20 0 L6 AND L19

=> d his

(FILE 'HOME' ENTERED AT 10:42:07 ON 14 MAY 2000)

FILE 'MEDLINE, BIOSIS, BIOTECHNO' ENTERED AT 10:43:03 ON 14 MAY 2000

```
L1          3206 S HIGH THROUGHPUT
L2         50715 S REPORTER
L3        188856 S PROMOTER
L4            22 S L1 AND L2 AND L3
L5            10 DUP REM L4 (12 DUPLICATES REMOVED)
L6           117 S L1 AND L2
L7             0 S L6 AND AMPLIF
L8        316071 S AMPLIF? OR COOPERATIVE OR SYNERGIS?
L9             8 S L8 AND L6
L10            4 DUP REM L9 (4 DUPLICATES REMOVED)
L11          109 S L6 NOT L9
L12           11 S L6 AND (ALL OR NONE)
L13            3 S L6 AND MAP
L14            5 S L6 AND MAP?
L15            4 DUP REM L14 (1 DUPLICATE REMOVED)
L16            5 S AUTOPOSITIVE
L17         1162 S FEEDFORWARD
L18         4220 S POSITIVE FEEDBACK
L19         5377 S L16 OR L17 OR L18
L20            0 S L6 AND L19

=> s l1 and l19

L21            1 L1 AND L19

=> d ibib abs


L21  ANSWER 1 OF 1  MEDLINE
ACCESSION NUMBER:   97268279     MEDLINE
DOCUMENT NUMBER:    97268279
TITLE:              VLSI neural system architecture for finite ring recursive
                    reduction.
AUTHOR:             Zhang D; Jullien G A
CORPORATE SOURCE:   Department of Computer Science, City University of Hong
                    Kong, Kowloon, Hong Kong.. dapeng@cs.cityu.edu.hk
SOURCE:             INTERNATIONAL JOURNAL OF NEURAL SYSTEMS, (1996 Dec) 7 (6)
                    697-708.
                    Journal code: BY2. ISSN: 0129-0657.
PUB. COUNTRY:       Singapore
                    Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        199708
ENTRY WEEK:         19970804
AB   The use of neural-like networks to implement finite ring computations has
     been presented in a previous paper. This paper develops efficient VLSI
     neural system architecture for the finite ring recursive reduction
(FRRR),
     including module reduction, MSB carry iteration and feedforward
     processing. These techniques deal with the basic principles involved in
     constructing a FRRR, and their implementations are efficiently matched to
     the VLSI medium. Compared with the other structure models for finite ring
     computation (e.g. modification of binary arithmetic logic and bit-steered
     ROM's), the FRRR structure has the lowest area complexity in silicon
while
     maintaining a high throughput rate. Examples of
     several implementations are used to illustrate the effectiveness of the
     FRRR architecture.


=> d his

     (FILE 'HOME' ENTERED AT 10:42:07 ON 14 MAY 2000)
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      FILE 'MEDLINE, [redacted]OSIS, BIOTECHNO' ENTERED AT 10:[redacted].03 ON 14 MAY 2000
L1          3206 S HIGH THROUGHPUT
L2         50715 S REPORTER
L3        188856 S PROMOTER
L4            22 S L1 AND L2 AND L3
L5            10 DUP REM L4 (12 DUPLICATES REMOVED)
L6           117 S L1 AND L2
L7             0 S L6 AND AMPLIF
L8        316071 S AMPLIF? OR COOPERATIVE OR SYNERGIS?
L9             8 S L8 AND L6
L10            4 DUP REM L9 (4 DUPLICATES REMOVED)
L11          109 S L6 NOT L9
L12           11 S L6 AND (ALL OR NONE)
L13            3 S L6 AND MAP
L14            5 S L6 AND MAP?
L15            4 DUP REM L14 (1 DUPLICATE REMOVED)
L16            5 S AUTOPOSITIVE
L17         1162 S FEEDFORWARD
L18         4220 S POSITIVE FEEDBACK
L19         5377 S L16 OR L17 OR L18
L20            0 S L6 AND L19
L21            1 S L1 AND L19

=> s screen

L22        45205 SCREEN

=> s l22 and l21

L23            0 L22 AND L21

=> s l22 and l19

L24           18 L22 AND L19

=> dup rem l24

PROCESSING COMPLETED FOR L24
L25           10 DUP REM L24 (8 DUPLICATES REMOVED)

=> d ibib abs 1-10


L25  ANSWER 1 OF 10  BIOSIS  COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER:    2000:184055  BIOSIS
DOCUMENT NUMBER:     PREV200000184055
TITLE:               Visual dominance in amending the directional parameter of
                    feedforward control.
AUTHOR(S):           Hirata, Chiaki (1); Yoshida, Shigeru
CORPORATE SOURCE:    (1) Doctoral Program in Health and Sport Sciences,
                     Institute of Health and Sport Sciences, University of
                     Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8574 Japan
SOURCE:              Journal of Motor Behavior, (March, 2000) Vol. 32, No. 1,
                     pp. 17-25.
                     ISSN: 0022-2895.
DOCUMENT TYPE:       Article
LANGUAGE:            English
SUMMARY LANGUAGE:    English
AB   The authors examined visual dominance between trials in which the
movement
     program was amended (i.e., off-line processing). Weighting between visual
     and proprioceptive feedback was examined in a trial-by-trial analysis of
     the directional parameter of feedforward control. Eight
     participants moved a cursor to a target displayed on a computer
     screen by manipulating a hand-held stylus on a digitizing tablet.
     In the first 30 trials, the cursor followed the stylus movement (practice
     condition). In the next 30 trials, the directional error of the stylus
     movement was presented in the opposite direction (reversal condition).
```

Subjects knew the presence and the nature of the reversal. In the last 10 trials, the reversal was withdrawn (transfer condition). Directional error of **feedforward** control was relatively small in the practice condition, and it increased gradually in 1 of 2 directions as trials proceeded in the reversal condition. Positive aftereffect was observed in the transfer condition. A constant increment of the directional error indicated that both visual and proprioceptive feedback are registered, with higher weight on vision, and that weighting between those inputs is determined automatically or is fixed without any strategic control.

L25 ANSWER 2 OF 10 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1999441136 MEDLINE
DOCUMENT NUMBER: 99441136
TITLE: Trigeminal deafferentation and conditioned pecking in pigeons.
AUTHOR: Bermejo R; Zeigler H P
CORPORATE SOURCE: Biopsychology Program, Hunter College (CUNY), New York City, New York 10021, USA.. hzeigler@shiva.hunter.cuny.edu
CONTRACT NUMBER: MH-00836 (NIMH)
K05-MH00320 (NIMH)
SOURCE: BEHAVIOURAL BRAIN RESEARCH, (1999 Mar) 99 (2) 181-9.
Journal code: AG3. ISSN: 0166-4328.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY WEEK: 20000104
AB To clarify the contribution of peripheral trigeminal input to the control of pecking behavior we examined head and jaw movement kinematics and peck localization in pigeons with surgical section of trigeminal nerves providing somatosensory input to the beak. Conditioning procedures were used to bring the pecking/grasping components of pecking under the control of a visual target. Conditioned head and jaw movements were monitored 'on-line' using movement transducers and terminal peck location was recorded using 'touch-**screen**' technology. The periodic delivery of a food reinforcer provided repeated opportunities to monitor the kinematics of ingestive pecks. Deafferentation produced deficits in mandibulation during ingestive pecking and in the coordination of head and jaw movements during conditioned pecking. These results are attributed to disruptions in trigeminal feedback and **feedforward** mechanisms, respectively. In contrast with previous studies, deafferentation did not impair the precision of peck localization. Possible reasons for the absence of localization deficits are presented. The results are discussed in relation to the role of peripheral inputs in the control of prehensile movements.

L25 ANSWER 3 OF 10 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1999149942 MEDLINE
DOCUMENT NUMBER: 99149942
TITLE: Impaired movement control in Alzheimer's disease.
AUTHOR: Ghilardi M F; Alberoni M; Marelli S; Rossi M; Franceschi M;
Ghez C; Fazio F
CORPORATE SOURCE: INB-CNR, Milano; Istituto Scientifico San Raffaele, Universita di Milano, Milan, Italy.. mg79@columbia.edu
CONTRACT NUMBER: K08NS01961 (NINDS)
NS22715 (NINDS)
SOURCE: NEUROSCIENCE LETTERS, (1999 Jan 22) 260 (1) 45-8. Ref: 20
Journal code: N7N. ISSN: 0304-3940.
PUB. COUNTRY: Ireland
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 9908
ENTRY WEEK: 990804

AB Movement accuracy in normal subjects depends on **feedforward** commands based on representation in memory of spatial and biomechanical features. Here we ask whether memory deficits in Alzheimer's disease (AD) interfere with movement planning and execution. Nine AD patients and nine age-matched controls moved a cursor to targets without seeing their limb. Starting and target positions were always visible on a **screen**, while, during movement, cursor position was either visible or blanked. Patients' paths showed discontinuous segments and prolonged movement time; movement inaccuracy, which increased without visual feedback, correlated significantly with scores of disease severity, working memory and attention.

L25 ANSWER 4 OF 10 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1997:312945 BIOSIS
DOCUMENT NUMBER: PREV199799603433
TITLE: Studies on mathematical model for predicting maize (Zea mays L.) production.
AUTHOR(S): Tsuiki, Mikinori
CORPORATE SOURCE: Project Res. Team 6, Natl. Agric. Res. Cent., 3-1-1 Kannondai, Tsukuba, Ibaraki 305 Japan
SOURCE: Sochi Shikenjo Kenkyu Hokoku, (1996) Vol. 0, No. 54, pp. 81-119.
ISSN: 0385-0196.
DOCUMENT TYPE: Article
LANGUAGE: Japanese
SUMMARY LANGUAGE: Japanese; English

AB The ability to predict maize (Zea mays L.) production is important to its management. Three different technologies are utilized to do this :sensing or monitoring current conditions, plant growth modeling, and predicting environmental changes. In this study, the ability to predict maize production by combining the simulation model and the meteorological pattern prediction with neural networks was considered. In Chapter 1, Continuous System Simulation Program, CSSP, was developed to help writing and executing dynamic models. Principal functions of CSSP were as follows:

1. Wherever there is a personal computer, CSSP can be used for making model. 2. As CSSP is an interactive program, it is suitable for try-and-error processes. 3. As CSSP was written in BASIC, users can execute simulations easier than others written in FORTRAN. 4. All the calculations in models were executed using the double precision for minimizing the accumulation of errors. 5. The most suitable integration method can be selected from three numerical methods, such as Runge-Kutta, Trapezoidal and Euler method. 6.Graphical output was available on CRT **screen** and/or printer. 7.As results of simulations were written in files, they can be used as data of other calculations. In Chapter 2, a mathematical model of maize was developed to simulate the growth under different meteorological conditions. CSSP was used to make this model. Developmental stages were estimated by non-parametric method. Dry matter weight of leaf, stem, ear and underground part was simulated on a daily time step. Mean air temperature and global solar radiation were considered as meteorological factors. In Chapter 3, the maize productivity in cool summer was evaluated using the model made in Chapter 2. The energy conversion efficiencies (yield/global solar radiation) for all growth period were almost the same in normal year and cool summer year. In Chapter 4, the program to construct multi-layer **feedforward** neural networks, NEUR092, was developed. Neural networks have been applied to pattern recognition problems. And they can also be applied to multivariate analysis, such as multiple regression analysis, discriminant analysis, principal component analysis and so on. NEUR092 has features as follows: 1.NEUR092 constructs multi-layer **feedforward** networks by using the most popular learning algorithm, back-propagation. 2. As the number of layers can be increased up to five, this program can be applied to many kinds of problems. 3. Simulated annealing algorithm is introduced to escape from local minimums. 4. Weight and offset values after learning

are written to file, and these data can be used as initial values of next approach. It will be possible to create a model of plant or animal growth using a neural network, as well as to make accurate estimates concerning grassland production. In Chapter 5, a neural network was applied to predicting meteorological patterns. The seasonal changes in 10-day mean air temperature monitored over a period of 45 years (from 1944 to 1988) were divided into 5 typical patterns. Neural networks were used to predict these patterns by 10-day mean air temperature till July. The maize production was expected to be nearly equal to that of a normal year in 1989, and higher than that of a normal year in 1990 and 1991 by using the maize simulation model and the neural network. The real maize production in and around National Grassland Research Institute agreed with these results.

L25 ANSWER 5 OF 10 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 96369239 MEDLINE
DOCUMENT NUMBER: 96369239
TITLE: Evidence of a limited visuo-motor memory used in programming wrist movements.
AUTHOR: Miall R C; Haggard P N; Cole J D
CORPORATE SOURCE: University Laboratory of Physiology, Oxford, UK.. rcm@physiol.ox.ac.uk
SOURCE: EXPERIMENTAL BRAIN RESEARCH, (1995) 107 (2) 267-80. Journal code: EP2. ISSN: 0014-4819.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612

AB Human subjects can pre-program movements on the basis of visual cues. Experience in a particular task leads to the storage of appropriate control parameters which are used in programming subsequent movements, via a short-term motor memory. The form, duration and usage of this memory are, however, uncertain. Repetitive wrist flexion and extension movements were measured in four subjects. Three were neurologically normal men; the fourth subject had a peripheral large-fibre sensory neuropathy, depriving him of proprioceptive information about wrist movement. Subjects made alternating 45 degrees wrist movements between two visual targets; visual feedback of wrist position was provided for the first part of each trial. After 10 s of tracking, the subjects paused for an interval of 0-24 s before resuming tracking without visual feedback of wrist position. The positional accuracy of subsequent movements was analysed with respect to pause interval. Movement accuracy was reduced by the removal of visual feedback in all four subjects: movements after the pause interval were less accurate than those before the pause. Errors also accumulated within each sequence of movements made without visual feedback. Analysis of the first movement in each trial after the pause indicated a clear relationship between movement accuracy and pause interval. In all four subjects, movement accuracy decayed with longer pause intervals. In the deafferented subject, manipulation of the visual inputs (requiring visual fixation, rather than normal pursuit of the target; or direct viewing of the hand instead of viewing a cursor on a computer **screen**) affected the relationship between pause interval and subsequent movement accuracy. We propose that the memory used when producing these movements is a short-lasting visuo-motor signal, lasting a few seconds, which is derived from visual knowledge of previous movements, rather than a memory of a particular motor output. This visuo-motor signal is used to scale the amplitude of subsequent wrist movements. The brevity of the visuo-motor memory and the resultant inaccuracy of this deafferented subject and of our neurologically normal subjects implies that human **feedforward** control of the amplitude and position of wrist movements is severely limited.

L25 ANSWER 6 OF 10 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 93236484 MEDLINE

DOCUMENT NUMBER: [redacted]36484
TITLE: [redacted]astric muscle response as a fu[redacted]ion of knowledge of the
task to be performed.
AUTHOR: van Willigen J D; Broekhuijsen M L; Melchior H J; Karkazis H C; Kossioni A; Heath M R
CORPORATE SOURCE: Department of Neurobiology and Oral Physiology, University of Groningen, The Netherlands.
SOURCE: ARCHIVES OF ORAL BIOLOGY, (1993 Feb) 38 (2) 113-21.
Journal code: 83M. ISSN: 0003-9969.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Dental Journals
ENTRY MONTH: 199307

AB Whether the motor programme executed by the digastric muscles during a forceful bite is modified according to a subject's expectation that the resistance between the teeth will change was investigated. There were two experimental conditions: (1) tracking a ramp (drawn on an oscilloscope **screen**) by biting (isometrically) on a force transducer and holding it at 120 N, and (2) tracking the same ramp with a sudden unloading at 100 N. There were two groups of experiments: (1) control experiments in which subjects underwent a sudden and unexpected unloading of the jaw, and (2) experiments in which subjects were previously informed whether or not there was to be an unloading. In all experiments the subjects co-contracted their digastric muscles during the bite as compared to the state at rest. The subjects' responses fell into the three different types: (i) those who varied the level of tonic digastric activity only as a function of the experimental condition, (ii) those who co-contracted the digastric muscles at the same time as the masseter muscles, and (iii) those who changed the contraction pattern of the digastric muscles as a function of the experimental condition. If modulation of the digastric muscles occurred this is a '**feedforward**' strategy mainly based on immediate past performance.

L25 ANSWER 7 OF 10 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 92265774 MEDLINE
DOCUMENT NUMBER: 92265774
TITLE: Artificial neural network classification of Drosophila courtship song mutants.
AUTHOR: Neumann E K; Wheeler D A; Bernstein A S; Burnside J W; Hall J C
CORPORATE SOURCE: Department of Biology, Brandeis University, Waltham, MA 02254..
CONTRACT NUMBER: GM-21473 (NIGMS)
SOURCE: BIOLOGICAL CYBERNETICS, (1992) 66 (6) 485-96.
Journal code: A2H. ISSN: 0340-1200.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199208

AB Courtship songs produced by Drosophila males--wild-type, plus the cacophony and dissonance behavioral mutants--were examined with the aid of newly developed strategies for adaptive acoustic analysis and classification. This system used several techniques involving artificial neural networks (a.k.a. parallel distributed processing), including learned vector quantization of signals and non-linear adaption (back-propagation) of data analysis. "Pulse" song from several individual wild-type and mutant males were first vector-quantized according to their frequency spectra. The accumulated quantized data of this kind, for a given song, were then used to "teach" or adapt a multiple-layered **feedforward** artificial neural network, which classified that song according to its original genotype. Results are presented on the performance of the final adapted system when faced with novel test data

and on acoustic features the system decides upon for predicting the song-mutant genotype in question. The potential applications and extensions of this new system are discussed, including how it could be used to **screen** for courtship mutants, search novel behavior patterns or cause-and-effect relationships associated with reproduction, compress these kinds of data for digital storage, and analyze Drosophila behavior beyond the case of courtship song.

L25 ANSWER 8 OF 10 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 91372370 MEDLINE
DOCUMENT NUMBER: 91372370
TITLE: Scaling of the metrics of visually-guided arm movements during motor learning in primates.
AUTHOR: Ojakangas C L; Ebner T J
CORPORATE SOURCE: Department of Neurosurgery, University of Minnesota, Minneapolis 55455..
CONTRACT NUMBER: NS-18338 (NINDS)
SOURCE: EXPERIMENTAL BRAIN RESEARCH, (1991) 85 (2) 314-23. Journal code: EP2. ISSN: 0014-4819.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199112
AB Hand trajectory, tangential velocity and acceleration, time and distance until peak velocity and reaction time were analyzed during the process of learning a skilled, visually-guided arm movement. Primates were trained to move a cursor with a manipulandum from a start box to target boxes displayed on a horizontal video **screen** during control conditions and when the relationship (gain) between the cursor and manipulandum was altered. The animals adapted to the altered feedback over 100-200 trials. A subsequent testing phase with randomly interspersed trials using the control gain demonstrated that the animals had modified their movements appropriately for the novel gain. Examination of the kinematics revealed that in adapting to a novel gain, primates scaled movement amplitude, tangential velocity, acceleration, and duration appropriately for the distance the hand needed to travel. Yet time to peak velocity was kept constant. Reaction time also remained unchanged for three of the four animals. Movements were performed in two phases, the first from movement onset to peak velocity and the second from peak velocity until the end of the movement. During the first phase the shape of the trajectory and velocity profile were stereotypic and without evidence of any corrections, consistent with this phase being essentially open loop. However, corrections occurred in the second phase and we propose visual feedback was used to correct for the difference in hand/cursor position. Learning appeared to involve utilizing the errors from previous trials to modify the early **feedforward** phase of subsequent trials. Peak tangential velocity, total movement duration and distance reached at peak tangential velocity all scaled linearly with the total movement distance required at each gain. Based on regression analyses, for none of these variables were the changes in learning completely adequate to compensate for total distance required. However, distance to peak velocity scaled with peak velocity in relation to the control gain. The results show that non-human primates adopt a consistent strategy when learning to scale a multi-joint movement. The metrics of the movement scaled yet the time to peak velocity remained constant, suggesting independent control of time and amplitude. Keeping time to peak velocity constant as well as the scaling of peak velocity with distance to peak velocity are viewed as ways to simplify the learning process.

L25 ANSWER 9 OF 10 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 86125469 MEDLINE
DOCUMENT NUMBER: 86125469
TITLE: [Hemispheric lateralization of visuo-spatial function in man].
O polusharnoi lateralizatsii zritel'no-prostranstvennoi

[redacted]ktsii u cheloveka.
AUTHOR: [redacted]tandov E A; Ivashchenko O I; V[redacted]ova T N
SOURCE: ZHURNAL VYSSHEI NERVNOI DEIATELNOSTI IMENI I. P. PAVLOVA, (1985 Nov-Dec) 35 (6) 1030-8.
Journal code: YAS. ISSN: 0044-4677.
PUB. COUNTRY: USSR
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Russian
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198605
AB Two parts of a geometrical figure are consecutively presented to healthy adult subjects in the left and right visual fields; the subjects have to compare them mentally and to decide whether these parts form a standard figure or not. Correctness of the reaction is controlled by a computer which lights up on the **screen** the words "good" or "error". The number of correct decisions of this visual-spatial task does not depend on the hemisphere to which information is addressed. The reaction time is substantially shorter if the information comes "directly" to the right hemisphere. Due to better training in the left hemisphere interhemispheric difference in reaction time gradually disappears in repeated tests. Training to mental "constructing" takes place only in the tests following **positive feedback** stimulus. Analysis of amplitude-temporal parameters of P300 wave shows that at correct decision of the visual-spatial task the level of activation in the right hemisphere is higher than in the left one.

L25 ANSWER 10 OF 10 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 81208398 MEDLINE
DOCUMENT NUMBER: 81208398
TITLE: A model of visual perception.
AUTHOR: Borello L; Ferraro M; Penengo P; Rossotti M L
SOURCE: BIOLOGICAL CYBERNETICS, (1981) 39 (2) 79-85.
Journal code: A2H. ISSN: 0340-7200.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198110
AB In this paper we propose a model of visual perception in which a **positive feedback** mechanism can reproduce the pattern stimulus on a neurons **screen**. The pattern stimulus reproduction is based on informations coming from the spatial derivatives of visual pattern. This information together with the response of the feature extractors provides to the reproduction of the visual pattern as neuron **screen** electric activity. We simulate several input patterns and prove that the model reproduces the percept.

=> d his

(FILE 'HOME' ENTERED AT 10:42:07 ON 14 MAY 2000)

FILE 'MEDLINE, BIOSIS, BIOTECHNO' ENTERED AT 10:43:03 ON 14 MAY 2000
L1 3206 S HIGH THROUGHPUT
L2 50715 S REPORTER
L3 188856 S PROMOTER
L4 22 S L1 AND L2 AND L3
L5 10 DUP REM L4 (12 DUPLICATES REMOVED)
L6 117 S L1 AND L2
L7 0 S L6 AND AMPLIF
L8 316071 S AMPLIF? OR COOPERATIVE OR SYNERGIS?
L9 8 S L8 AND L6
L10 4 DUP REM L9 (4 DUPLICATES REMOVED)
L11 109 S L6 NOT L9
L12 11 S L6 AND (ALL OR NONE)

```
L13           3 S L6 [redacted]D MAP
L14           5 S L6 [redacted]D MAP?
L15           4 DUP REM L14 (1 DUPLICATE REMOVED)
L16           5 S AUTOPOSITIVE
L17        1162 S FEEDFORWARD
L18        4220 S POSITIVE FEEDBACK
L19        5377 S L16 OR L17 OR L18
L20           0 S L6 AND L19
L21           1 S L1 AND L19
L22       45205 S SCREEN
L23           0 S L22 AND L21
L24          18 S L22 AND L19
L25          10 DUP REM L24 (8 DUPLICATES REMOVED)

=> s assay

L26      622407 ASSAY

=> s l26 and l19

L27         117 L26 AND L19

=> s l2 and l27

L28           7 L2 AND L27

=> d ibib abs 1-7

L28  ANSWER 1 OF 7  MEDLINE
ACCESSION NUMBER:   1999009119     MEDLINE
DOCUMENT NUMBER:    99009119
TITLE:              Homologous up-regulation of KDR/Flk-1 receptor expression
                    by vascular endothelial growth factor in vitro.
AUTHOR:             Shen B Q; Lee D Y; Gerber H P; Keyt B A; Ferrara N;
                    Zioncheck T F
CORPORATE SOURCE:   Department of Pharmacokinetics and Metabolism, Genentech,
                    Inc., South San Francisco, California 94080, USA.
SOURCE:             JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Nov 6) 273 (45)
                    29979-85.
                    Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY:       United States
                    Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals; Cancer Journals
ENTRY MONTH:        199902
AB   We investigated the possibility that vascular endothelial growth factor
     (VEGF) treatment could regulate KDR/Flk-1 receptor expression in
     endothelial cells. Bovine adrenal cortex endothelial cells were incubated
     with 200 pM rhVEGF165 for 0-7 days. Western blot analysis showed a
     3-5-fold increase in total KDR protein following 4-day VEGF treatment.
     Scatchard analysis revealed that VEGF induced a 2-3-fold increase in high
     affinity receptor number (5.0 x 10(4)/cell versus 2. 4 x 10(4)/cell)
     without significantly affecting receptor binding affinity (Kd 76 pM
versus
     72 pM). Quantitative polymerase chain reaction analysis demonstrated a
     3-fold increase in KDR mRNA levels following VEGF exposure. VEGF-induced
     KDR expression primarily occurred at the transcriptional level as
     demonstrated by a luciferase reporter assay system.
     Receptor selective mutants with wild-type KDR binding and decreased Flt-1
     binding also induced KDR up-regulation; in contrast, mutants with
     decreased KDR binding and wild-type Flt-1 binding did not, suggesting
that
     KDR receptor signaling mediated the increase in KDR expression.
Inhibition
     of tyrosine kinase, Src tyrosine kinase, protein kinase C, and
     mitogen-activated protein kinase activities all blocked VEGF-induced KDR
     up-regulation. Finally, co-incubation of nitric-oxide synthase inhibitors
     with VEGF had no significant effect on KDR expression, but 100 microM
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sodium nitroprusside, a NO donor, significantly inhibited VEGF-induced KDR up-regulation, indicating that NO negatively regulates KDR expression. In conclusion, our data demonstrate that VEGF binding to the KDR receptor tyrosine kinase results in an increase in KDR receptor gene transcription and protein expression. Thus, KDR up-regulation induced by VEGF may represent an important **positive feedback** mechanism for VEGF action in tumor and ischemia-induced angiogenesis.

L28 ANSWER 2 OF 7 MEDLINE
ACCESSION NUMBER: 1998373896 MEDLINE
DOCUMENT NUMBER: 98373896
TITLE: Hypoxia induces high-mobility-group protein I(Y) and transcription of the cyclooxygenase-2 gene in human vascular endothelium.
AUTHOR: Ji Y S; Xu Q; Schmedtje J F Jr
CORPORATE SOURCE: Sealy Center for Molecular Cardiology, Department of Medicine, The University of Texas Medical Branch, Galveston, USA.
SOURCE: CIRCULATION RESEARCH, (1998 Aug 10) 83 (3) 295-304. Journal code: DAJ. ISSN: 0009-7330.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY WEEK: 19981101

AB Cyclooxygenases catalyze a rate-limiting step in the synthesis of vascular endothelial prostaglandins. Expression of the inducible cyclooxygenase-2 (COX-2) gene is increased by hypoxia in human vascular endothelial cells via the nuclear factor (NF)-kappaB p65 transcription factor, which is necessary but not sufficient to fully induce COX-2 transcription in response to hypoxia. After finding that cytoplasmic NF-kappaB p65 and IkappaBalpha (an inhibitory protein that binds NF-kappaB p65 precursors) levels are not changed by hypoxia, we hypothesized that other factors might play a role in regulating the COX-2 promoter, like the high-mobility-group (HMG) I(Y) family of proteins, which features multiple A.T hooks and is associated with NF-kappaB-mediated transactivation. Nuclear protein obtained from human umbilical vein endothelial cells (HUVECs) was supplemented with HMG I(Y) during electrophoretic mobility shift assays using an NF-kappaB-3' element probe. These data suggested that HMG I(Y) proteins interact with NF-kappaB p65 to induce COX-2 promoter activity. We also found that TATA-box DNA demonstrated increased electrophoretic shifting indicative of DNA binding after incubation with either hypoxic HUVEC nuclear protein or normoxic nuclear protein supplemented with HMG I(Y). Transfection of HUVECs with an expression vector containing the COX-2 promoter ligated to HMG I(Y) cDNA demonstrated **positive feedback** on COX-2 promoter activity in hypoxia. We confirmed that COX-2 is transcriptionally regulated by hypoxia using a nuclear runoff **assay**. Hypoxia increased steady-state cellular levels of HMG I(Y) mRNA as an early event, corresponding with increases in HMG I(Y) protein. Overexpression of HMG I(Y) was associated in a dose-response relationship with increasing prevalence of the COX-2 protein in hypoxic HUVECs. Furthermore, sense (and antisense) HMG I(Y) overexpression caused stimulation (or inhibition) of COX-2 promoter activity as measured by luciferase **reporter** gene expression. The physiological significance of these findings was demonstrated by cyclooxygenase-dependent release of prostaglandin E2 by HUVECs in hypoxia. We concluded that hypoxia increases expression of HMG I(Y) proteins while facilitating transactivation of the COX-2 promoter. The HMG I(Y) family of proteins may therefore function as part of a hypoxia-induced enhanceosome that helps to promote transcription of COX-2.

L28 ANSWER 3 OF 7 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1999:4973 BIOSIS
DOCUMENT NUMBER: PREV199900004973
TITLE: Homologous up-regulation of KDR/Flk-1 receptor expression by vascular endothelial growth factor in vitro.
AUTHOR(S): Shen, Ben-Quan; Lee, David Y.; Gerber, Hans-Peter; Keyt, Bruce A.; Ferrara, Napoleone; Zioncheck, Thomas F. (1)
CORPORATE SOURCE: (1) Dep. Pharmacokinetics Metabolism, Genentech Inc., MS 70, 1 DNA Way, South San Francisco, CA 94080 USA
SOURCE: Journal of Biological Chemistry, (Nov. 6, 1998) Vol. 273, No. 45, pp. 29979-29985.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB We investigated the possibility that vascular endothelial growth factor (VEGF) treatment could regulate KDR/Flk-1 receptor expression in endothelial cells. Bovine adrenal cortex endothelial cells were incubated with 200 pM rhVEGF165 for 0-7 days. Western blot analysis showed a 3-5-fold increase in total KDR protein following 4-day VEGF treatment. Scatchard analysis revealed that VEGF induced a 2-3-fold increase in high affinity receptor number (5.0 X 104/cell versus 2.4 X 104/ cell) without significantly affecting receptor binding affinity (Kd 76 pM versus 72 pM).
Quantitative polymerase chain reaction analysis demonstrated a 3-fold increase in KDR mRNA levels following VEGF exposure. VEGF-induced KDR expression primarily occurred at the transcriptional level as demonstrated
by a luciferase **reporter assay** system. Receptor
selective mutants with wild-type KDR binding and decreased Flt-1 binding also induced KDR up-regulation; in contrast, mutants with decreased KDR binding and wild-type Flt-1 binding did not, suggesting that KDR receptor signaling mediated the increase in KDR expression. Inhibition of tyrosine kinase, Src tyrosine kinase, protein kinase C, and mitogen-activated protein kinase activities all blocked VEGF-induced KDR up-regulation. Finally, co-incubation of nitric-oxide synthase inhibitors with VEGF had no significant effect on KDR expression, but 100 muM sodium nitroprusside,
a NO donor, significantly inhibited VEGF-induced KDR up-regulation, indicating that NO negatively regulates KDR expression. In conclusion, our
data demonstrate that VEGF binding to the KDR receptor tyrosine kinase results in an increase in KDR receptor gene transcription and protein expression. Thus, KDR up-regulation induced by VEGF may represent an important **positive feedback** mechanism for VEGF action
in tumor and ischemia-induced angiogenesis.

L28 ANSWER 4 OF 7 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1998:503036 BIOSIS
DOCUMENT NUMBER: PREV199800503036
TITLE: Inducible cAMP early repressor ICER down-regulation of CREB
gene expression in Sertoli cells.
AUTHOR(S): Walker, William H.; Daniel, Philip B.; Habener, Joel F. (1)
CORPORATE SOURCE: (1) Lab. Mol. Endocrinol., Mass. Gen. Hosp., Howard Hughes Med. Inst., Harvard Med. Sch., Wellman 320, Boston, MA 02114 USA
SOURCE: Molecular and Cellular Endocrinology, (Aug. 25, 1998) Vol. 143, No. 1-2, pp. 167-178.
ISSN: 0303-7207.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The cAMP response element binding protein (CREB) and the cAMP-responsive element modulator (CREM) are cyclically expressed in the seminiferous tubules during spermatogenesis. In the somatic Sertoli cells, which are the major supporters of germ cell development in the seminiferous tubules,
the expression of CREB is cyclical and appears to be regulated by the

levels of cAMP induced in response to the pituitary derived follicle-stimulating hormone FSH. Cyclic AMP response elements (CREs) located in the promoter of the CREB gene were shown earlier to be implicated in an **autopositive** feedback loop that up-regulates the expression of CREB. Here we show that in Sertoli cells FSH-mediated induction of the CREM repressor isoform, ICER (inducible cAMP early repressor) is correlated with the inhibition and delay of CREB gene expression in the seminiferous tubules. ICER binds to the two CREs located in the promoter of the CREB gene and in transient transfection assays of Sertoli cells, ICER expression vectors down-regulate transcription of a **reporter** gene driven by the CREB gene promoter. In addition, analyses of ICER and CREB gene expression in isolated segments of rat seminiferous tubules reveals stage-specific and cycle-dependent expression of ICER. The periods of enhanced expression of ICER correspond to the stages of spermatogenesis with the lowest levels of CREB expression. We suggest that the expression of ICER in Sertoli cells may contribute to the periodic repression of CREB gene expression during the repeated 12-day cycles of spermatogenesis, and may be required to reset the levels of activator CREB prior to the initiation of each new cycle of spermatogenesis.

L28 ANSWER 5 OF 7 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1998:429437 BIOSIS
DOCUMENT NUMBER: PREV199800429437
TITLE: Hypoxia induces high-mobility-group protein I(Y) and transcription of the cyclooxygenase-2 gene in human vascular endothelium.
AUTHOR(S): Ji, Yan-Shan; Xu, Qing; Schmedtje, John F., Jr. (1)
CORPORATE SOURCE: (1) Section Cardiol., Wake Forest Univ. Sch. Med., Medical Center Blvd., Winston-Salem, NC 27157 USA
SOURCE: Circulation Research, (Aug., 1998) Vol. 83, No. 3, pp. 295-304.
ISSN: 0009-7330.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Cyclooxygenases catalyze a rate-limiting step in the synthesis of vascular endothelial prostaglandins. Expression of the inducible cyclooxygenase-2 (COX-2) gene is increased by hypoxia in human vascular endothelial cells via the nuclear factor (NF)-kappaB p65 transcription factor, which is necessary but not sufficient to fully induce COX-2 transcription in response to hypoxia. After finding that cytoplasmic NF-kappaB p65 and IkappaBalpha (an inhibitory protein that binds NF-kappaB p65 precursors) levels are not changed by hypoxia, we hypothesized that other factors might play a role in regulating the COX-2 promoter, like the high-mobility-group (HMG) I(Y) family of proteins, which features multiple A-T hooks and is associated with NF-kappaB-mediated transactivation. Nuclear protein obtained from human umbilical vein endothelial cells (HUVECs) was supplemented with HMG I(Y) during electrophoretic mobility shift assays using an NF-kappaB-3' element probe. These data suggested that HMG I(Y) proteins interact with NF-kappaB p65 to induce COX-2 promoter activity. We also found that TATA-box DNA demonstrated increased electrophoretic shifting indicative of DNA binding after incubation with either hypoxic HUVEC nuclear protein or normoxic nuclear protein supplemented with HMG I(Y). Transfection of HUVECs with an expression vector containing the COX-2 promoter ligated to HMG I(Y) cDNA demonstrated **positive feedback** on COX-2 promoter activity in hypoxia. We confirmed that COX-2 is transcriptionally regulated by hypoxia using a nuclear runoff **assay**. Hypoxia increased steady-state cellular levels of HMG I(Y) mRNA as an early event, corresponding with increases in HMG I(Y) protein. Overexpression of HMG I(Y) was associated in a dose-response relationship with increasing prevalence of the COX-2 protein

in hypoxic HUVE[redacted] Furthermore, sense (and antis[redacted]e) HMG I(Y) overexpression [redacted]sed stimulation (or inhibition[redacted]f COX-2 promoter activity as measured by luciferase **reporter** gene expression. The physiological significance of these findings was demonstrated by cyclooxygenase-dependent release of prostaglandin E2 by HUVECs in hypoxia.

We concluded that hypoxia increases expression of HMG I(Y) proteins while facilitating transactivation of the COX-2 promoter. The HMG I(Y) family of proteins may therefore function as part of a hypoxia-induced enhanceosome that helps to promote

L28 ANSWER 6 OF 7 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
ACCESSION NUMBER: 1998:28510016 BIOTECHNO
TITLE: Homologous up-regulation of KDR/Flk-1 receptor expression by vascular endothelial growth factor in vitro
AUTHOR: Shen B.-Q.; Lee D.Y.; Gerber H.-P.; Key B.A.; Ferrara N.; Zioncheck T.F.
CORPORATE SOURCE: T.F. Zioncheck, Dept. of Pharmacokinetics/Metabol., Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, United States.
E-mail: zioncheck.tom@gene.com
SOURCE: Journal of Biological Chemistry, (06 NOV 1998), 273/45
(29979-29985), 62 reference(s)
CODEN: JBCHA3 ISSN: 0021-9258
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28510016 BIOTECHNO
AB We investigated the possibility that vascular endothelial growth factor (VEGF) treatment could regulate KDR/Flk-1 receptor expression in endothelial cells. Bovine adrenal cortex endothelial cells were incubated with 200 pM rhVEGF.sub.1.sub.6.sub.5 for 0-7 days. Western blot analysis showed a 3-5-fold increase in total KDR protein following 4-day VEGF treatment. Scatchard analysis revealed that VEGF induced a 2-3-fold increase in high affinity receptor number (5.0 x 10.sup.4/cell versus 2.4 x 10.sup.4/ cell) without significantly affecting receptor binding affinity (K(d) 76 pM versus 72 pM). Quantitative polymerase chain reaction analysis demonstrated a 3-fold increase in KDR mRNA levels following VEGF exposure. VEGF-induced KDR expression primarily occurred at the transcriptional level as demonstrated by a luciferase **reporter assay** system. Receptor selective mutants with wild-type KDR binding and decreased Flt-1 binding also induced KDR up-regulation; in contrast, mutants with decreased KDR binding and wild-type Flt-1 binding did not, suggesting that KDR receptor signaling mediated the increase in KDR expression. Inhibition of tyrosine kinase, Src tyrosine kinase, protein kinase C, and mitogen-activated protein kinase activities all blocked VEGF- induced KDR up-regulation. Finally, co-incubation of nitric-oxide synthase inhibitors with VEGF had no significant effect on KDR expression, but 100.mu.M sodium nitroprusside, a NO donor, significantly inhibited VEGF-induced KDR up-regulation, indicating that NO negatively regulates KDR expression. In conclusion, our data demonstrate that VEGF binding to the KDR receptor tyrosine kinase results in an increase in KDR receptor gene transcription and protein expression. Thus, KDR up-regulation induced by VEGF may represent an important **positive feedback** mechanism for VEGF action in tumor and ischemia- induced angiogenesis.

L28 ANSWER 7 OF 7 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
ACCESSION NUMBER: 1998:28371319 BIOTECHNO
TITLE: Hypoxia induces high-mobility-group protein I(Y) and transcription of the cyclooxygenase-2 gene in human vascular endothelium

AUTHOR: Ji Y.-S.; Xu Q.; Schmedtje J[redacted] Jr.
CORPORATE SOURCE: Dr. J.F. Schmedtje Jr., Sect[redacted] on Cardiology, Wake Forest Univ. School Of Medicine, Medical Center Blvd, Winston-Salem, NC 27157, United States.
SOURCE: Circulation Research, (10 AUG 1998), 83/3 (295-304), 39 reference(s)
CODEN: CIRUAL ISSN: 0009-7330
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28371319 BIOTECHNO
AB Cyclooxygenases catalyze a rate-limiting step in the synthesis of vascular endothelial prostaglandins. Expression of the inducible cyclooxygenase-2 (COX-2) gene is increased by hypoxia in human vascular endothelial cells via the nuclear factor (NF)-.kappa.B p65 transcription factor, which is necessary but not sufficient to fully induce COX-2 transcription in response to hypoxia. After finding that cytoplasmic NF-.kappa.B p65 and I.kappa.B.alpha. (an inhibitory protein that binds NF-.kappa.B p65 precursors) levels are not changed by hypoxia, we hypothesized that other factors might play a role in regulating the COX-2 promoter, like the high-mobility-group (HMG) I(Y) family of proteins, which features multiple A.midldot.T hooks and is associated with NF-.kappa.B-mediated transactivation. Nuclear protein obtained from human umbilical vein endothelial cells (HUVECs) was supplemented with HMG I(Y) during electrophoretic mobility shift assays using an NF-.kappa.B-3' element probe. These data suggested that HMG I(Y) proteins interact with NF-.kappa.B p65 to induce COX- 2 promoter activity. We also found that TATA-box DNA demonstrated increased electrophoretic shifting indicative of DNA binding after incubation with either hypoxic HUVEC nuclear protein or normoxic nuclear protein supplemented with HMG I(Y). Transfection of HUVECs with an expression vector containing the COX-2 promoter ligated to HMG I(Y) cDNA demonstrated **positive feedback** on COX-2 promoter activity in hypoxia. We confirmed that COX-2 is transcriptionally regulated by hypoxia using a nuclear runoff **assay**. Hypoxia increased steady-state cellular levels of HMG I(Y) mRNA as an early event, corresponding with increases in HMG I(Y) protein.
Overexpression of HMG I(Y) was associated in a dose-response relationship with increasing prevalence of the COX-2 protein in hypoxic HUVECs. Furthermore, sense (and antisense) HMG I(Y) overexpression caused stimulation (or inhibition) of COX-2 promoter activity as measured by luciferase **reporter** gene expression. The physiological significance of these findings was demonstrated by cyclooxygenase-dependent release of prostaglandin E.sub.2 by HUVECs in hypoxia. We concluded that hypoxia increases expression of HMG I(Y) proteins while facilitating transactivation of the COX-2 promoter. The HMG I(Y) family of proteins may therefore function as part of a hypoxia-induced enhanceosome that helps to promote transcription of COX-2.

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FILE 'MEDLINE, BIOSIS, BIOTECHNO' ENTERED AT 10:43:03 ON 14 MAY 2000
L1 3206 S HIGH THROUGHPUT
L2 50715 S REPORTER
L3 188856 S PROMOTER
L4 22 S L1 AND L2 AND L3
L5 10 DUP REM L4 (12 DUPLICATES REMOVED)
L6 117 S L1 AND L2
L7 0 S L6 AND AMPLIF
L8 316071 S AMPLIF? OR COOPERATIVE OR SYNERGIS?

L9	8	S L8 [illegible] L6
L10	4	DUP [illegible] L9 (4 DUPLICATES REMOVED)
L11	109	S L6 NOT L9
L12	11	S L6 AND (ALL OR NONE)
L13	3	S L6 AND MAP
L14	5	S L6 AND MAP?
L15	4	DUP REM L14 (1 DUPLICATE REMOVED)
L16	5	S AUTOPOSITIVE
L17	1162	S FEEDFORWARD
L18	4220	S POSITIVE FEEDBACK
L19	5377	S L16 OR L17 OR L18
L20	0	S L6 AND L19
L21	1	S L1 AND L19
L22	45205	S SCREEN
L23	0	S L22 AND L21
L24	18	S L22 AND L19
L25	10	DUP REM L24 (8 DUPLICATES REMOVED)
L26	622407	S ASSAY
L27	117	S L26 AND L19
L28	7	S L2 AND L27

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FULL ESTIMATED COST	4.03	4.78

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L3 14 L1 AND L2

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L4 12 DUP REM L3 (2 DUPLICATES REMOVED)

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L4 ANSWER 1 OF 12 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-05311 BIOTECHDS
TITLE: Novel methods for inhibiting TEF-3 activity used to treat matrix metallo protease-mediated diseases; recombinant transcriptional enhancer factor-3-inhibitor production via vector-mediated gene transfer and expression in bacterium host cell for e.g. Lyme disease gene therapy
AUTHOR: Tindal M H; Wang R L
PATENT ASSIGNEE: Procter+Gamble
LOCATION: Cincinnati, OH, USA.
PATENT INFO: WO 2000000161 6 Jan 2000
APPLICATION INFO: WO 1999-US14829 30 Jun 1999
PRIORITY INFO: US 1998-91318 30 Jun 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2000-170882 [15]
AN 2000-05311 BIOTECHDS
AB A method for determining whether or not a compound is an inhibitor of transcriptional enhancer factor-3 (TEF-3) activity which consists of culturing cells containing a TEF-3 responsive gene which consists of a TEF-3 responsive gene promoter and a **reporter** cDNA, incubating the cells with a sample of the compound to be tested and the monitoring the cells for a response from the promoter, is new. Also claimed are: a method for producing recombinant TEF-3 in a bacterial or eukaryotic cell which involves transforming the host cells with a vector containing a TEF-3 gene; methods for determining whether a compound is an inhibitor of TEF-3 activity, including one which is especially a **high throughput** screening method; a TEF-3 interacting factor; an antibody specific for TEF-3; and a method for preventing or treating a matrix metallo protease (MMP)-mediated disorder such as degenerative joint disorder or osteoarthritis, which involves administering a TEF-3-inhibitor. The above may be useful for finding TEF-3-inhibitors which may be useful for the prevention and treatment (e.g. gene therapy) of disease such as rheumatoid arthritis, Lyme disease and hyaline cartilage diseases. (31pp)

L4 ANSWER 2 OF 12 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1998-07622 BIOTECHDS
TITLE: Detecting compounds that mimic or modulate the effects of

ob[redacted]cotein;
[redacted]achexia, obesity, anorexia and [redacted]abetes drug screening
AUTHOR: Beeley L J
PATENT ASSIGNEE: SK-Beecham
LOCATION: Brentford, UK.
PATENT INFO: WO 98200158 14 May 1998
APPLICATION INFO: WO 1997-GB2988 30 Oct 1997
PRIORITY INFO: GB 1996-22850 1 Nov 1996; GB 1996-22866 1 Nov 1996
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-286968 [25]
AN 1998-07622 BIOTECHDS
AB New methods for detecting a compound that mimics or potentiates or inhibits the physiological effect of the ob protein (or leptin) comprise assessing the effect of the compound on: an ob-protein activated signal transducer and activator of transcription (STAT) DNA response element coupled to a **reporter** gene; and the response provided by ob-protein on an ob-protein activated STAT DNA response element coupled to a **reporter** gene. The response element and the **reporter** are expressed in an ob-protein responsive cell line selected from hypothalamic, pheochromocytoma, hematopoietic, pancreatic, liver, preadipocyte, skeletal muscle or ovarian derived cell lines, alternatively, they are expressed in an engineered cell line which is also transfected with a polypeptide capable of stimulating an ob-protein activated STAT DNA response element and containing the appropriate STAT proteins. The method is useful in **high throughput** assays for compounds to treat weight, energy balance, hematopoietic, fertility and other disorders involving ob protein, especially disorders related to obesity, anorexia, cachexia and diabetes. (20pp)

L4 ANSWER 3 OF 12 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-02209 BIOTECHDS
TITLE: **High throughput** method for creating and screening recombinant adeno viruses; adeno virus screening for potential use in gene therapy and research
AUTHOR: Davis A R; Meyers K; *Wilson J M
CORPORATE SOURCE: Univ.Pennsylvania-Inst.Hum.Gene-Ther.
LOCATION: The Institute for Human Gene Therapy and Department of Cellular and Molecular Engineering, University of Pennsylvania, The Wistar Institute, 3600 Spruce Street, Philadelphia, PA 19104, USA.
SOURCE: Gene Ther.; (1998) 5, 8, 1148-52
CODEN: GETHEC
ISSN: 0969-7128
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1999-02209 BIOTECHDS
AB Replication-defective adeno viruses are potential vectors for gene therapy and research tools for studying gene function. Important to their successful use is development of a method to isolate new recombinants rapidly, which are not contaminated with wild-type virus. A modification of the traditional method to create recombinant adeno viruses in which a 5' plasmid containing vector sequence is cotransfected into 293 cells with virus DNA is described. In the method, the virus DNA is delivered from the 3' portion of an E1-deleted recombinant that expresses the green fluorescent protein. Visualization of the cotransfection by fluorescent microscopy distinguished the recombinant plaques (nonfluorescent or white plaque) from background plaques (green fluorescent or green plaque). Using this approach, the success and throughput for creating new recombinants were increased while minimizing contamination. This has been used to isolate adeno virus vectors deleted in a number of essential genes. (

L4 ANSWER 4 OF 12 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD

ACCESSION NUMBER: 1999-08516 BIOTECHDS
TITLE: Development of a green fluorescent protein **reporter** for a yeast genotoxicity biosensor;
DNA damage-stimulated yeast enhanced green fluorescent protein expression in Saccharomyces cerevisiae, useful for
drug screening, detecting DNA repair gene and environmental stress
AUTHOR: Billinton N; Barker M G; Michel C E; Knight A W; Heyer W D; Goddard N J; Fielden P R; *Walmsley R M
CORPORATE SOURCE: Univ.Manchester-Inst.Sci.Technol.
LOCATION: Department of Biomolecular Sciences and Department of Instrumentation and Analytical Science, UMIST, Manchester M60
1QD, UK.
Email: walmsley@umist.ac.uk
SOURCE: Biosensors Bioelectron.; (1998) 13, 7-8, 831-38
CODEN: BBIOE4
ISSN: 0956-5663
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1999-08516 BIOTECHDS
AB A **reporter** system was developed and described for use in a genotoxicity sensor to screen for new genes involved in DNA repair in Saccharomyces cerevisiae. If DNA damage has occurred, the strain produces yeast enhanced green fluorescent protein (GFP) by expression of plasmid pWDH443. The GFP was codon optimized for yeasts. The **reporter** effectively responds to the genetic regulation of DNA repair and does not respond to chemicals which delay mitosis. The improved strain demonstrates improved signal to noise ratio, convenient data collection and uncomplicated material handling. The combination of inducible promoter and GFP **reporter** allows environmental stress monitoring and drug screening by simple and continuous, **high throughput** biosensor technology. The generic technology of the **reporter** could be tailored to more specific sensing applications. (26 ref)

L4 ANSWER 5 OF 12 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1998-11053 BIOTECHDS
TITLE: Real-time fluorescence detection of a single DNA molecule; single copy gene detection using improved polymerase chain
reaction and fluorescence label
AUTHOR: Lockey C; Otto E; *Long Z
CORPORATE SOURCE: Genet.Ther.
LOCATION: Genetic Therapy Inc., A Novartis Company, 938 Clopper Road, Gaithersburg, MD 20878, USA.
Email: zhifeng.long@pharma.novartis.com
SOURCE: BioTechniques; (1998) 24, 5, 744-46
CODEN: BTNQDO
ISSN: 0736-6205
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1998-11053 BIOTECHDS
AB A method for the real-time detection of a single copy herpes simplex virus thymidine-kinase (EC-2.7.1.21) gene in a high background of genomic
DNA, equivalent to 500,000 diploid mammal cells, is described. The method
involved an improved polymerase chain reaction (PCR) protocol and validation strategy. Detection used the fluorescence-based system of the
ABI Model-7700. A 417 bp sequence was amplified and detected using a specified DNA primer set and a probe covalently linked with the **reporter** dye FAM and the quencher dye TAMRA. The amplification profile was: 50 deg for 2 min (enzyme degradation), 95 deg for 10 min (hot start) followed by 60 cycles at 95 deg for 15 sec and 60 deg for 1.5
min. The limit of detection was determined using standards (specified).

The results obtained were comparable with results obtained by agarose gel electrophoresis and Southern blot hybridization. The method is useful for **high-throughput** PCR and to detect low levels of viruses or rare mutations in a large number of normal cells or biological samples. (4 ref)

L4 ANSWER 6 OF 12 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1997-08188 BIOTECHDS
TITLE: New isolated promoter from the obesity gene and mixtures with C/EBP DNA binding protein; obesity gene promoter-mediated **reporter** gene expression in adipocyte cell culture for use in pharmaceutical lead compound drug screening, gene therapy and mutation detection for diagnosis
AUTHOR: de la Brousse F C; Chen J L
PATENT ASSIGNEE: Tularik
LOCATION: South San Francisco, CA, USA.
PATENT INFO: WO 97180228 22 May 1997
APPLICATION INFO: WO 1996-US18474 18 Nov 1996
PRIORITY INFO: US 1995-558545 16 Nov 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1997-289220 [26]
AN 1997-08188 BIOTECHDS
AB An isolated obesity gene transcriptional promoter is claimed, containing a C/EBP binding site and preferably a 5' untranslated obesity gene exon, and capable of hybridizing under low stringency conditions with specified DNA sequences. Also claimed are: (a) a mixture containing the promoter and a C/EBP; and (b) a method for identifying pharmaceutical lead compounds, which involves combining the mixture in (a) with a candidate agent under conditions where, but for the presence of the agent, the promoter and the C/EBP form a first association, and detecting the presence of a second association of the promoter and C/EBP, where a difference between the first and second association indicates the agent is a pharmaceutical lead compound. In particular, transfected adipocytes containing obesity gene promoters operably linked to a **reporter** gene are used in **high-throughput** drug screening methods. The transcriptional promoter may also be used in gene therapy, for detection of obesity gene mutations and to amplify/identify nucleic acid encoding analogs or homologs. (17pp)

L4 ANSWER 7 OF 12 BIOBUSINESS COPYRIGHT 2000 BIOSIS DUPLICATE 1
ACCESSION NUMBER: 96:41808 BIOBUSINESS
DOCUMENT NUMBER: 0806669
TITLE: Improved green fluorescent protein by molecular evolution using DNA shuffling.
AUTHOR: Crameri A; Whitehorn E A; Tate E; Stemmer W P C
CORPORATE SOURCE: Affymax Res. Inst., 4001 Miranda Ave., Palo Alto, CA 94304, USA
SOURCE: Nature Biotechnology, (1996) Vol.14, No.3, P.315-319. ISSN: 1087-0156.
FILE SEGMENT: NONUNIQUE
LANGUAGE: ENGLISH
AB Green fluorescent protein (GFP) has rapidly become a widely used **reporter** of gene regulation. However, for many organisms, particularly eukaryotes, a stronger whole cell fluorescence signal is desirable. We constructed a synthetic GFP gene with improved codon usage and performed recursive cycles of DNA shuffling followed by screening for the brightest E. coli colonies. A visual screen using UV light, rather than FACS selection, was used to avoid red-shifting the excitation maximum. After 3 cycles of DNA shuffling, a mutant was obtained with a whole cell fluorescence signal that was 45-fold greater than a standard, the commercially available Clontech plasmid pGFP. The expression level in

E. coli was unaffected at about 75% of total protein. The emission and excitation maxima were also unchanged. Whereas in E. coli most of the wildtype GFP ends up in inclusion bodies, unable to activate its chromophore, most of the mutant protein is soluble and active. Three amino acid mutations appear to guide the mutant protein into the native folding pathway rather than toward aggregation. Expressed in Chinese Hamster Ovary (CHO) cells, this shuffled GFP mutant showed a 42-fold improvement over wildtype GFP sequence, and is easily detected with UV light in a wide range of assays. The results demonstrate how molecular evolution can solve a complex practical problem without needing to first identify which process is limiting. DNA shuffling can be combined with screening of a moderate number of mutants. We envision that the combination of DNA shuffling and **high throughput** screening will be a powerful tool for the optimization of many commercially important enzymes for which selections do not exist.

L4 ANSWER 8 OF 12 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1995-07475 BIOTECHDS
TITLE: Detecting inhibitors of protein or mRNA biosynthesis; herbicide and antibiotic drug screening system involving beta-galactosidase **reporter** gene expression and in vitro transcription-translation with an Escherichia coli extract
AUTHOR: Hawkes T R
PATENT ASSIGNEE: Zeneca
PATENT INFO: WO 95090925 13 Apr 1995
APPLICATION INFO: WO 1994-GB2088 26 Sep 1994
PRIORITY INFO: GB 1993-20562 6 Oct 1993
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1995-155266 [20]
AN 1995-07475 BIOTECHDS
AB A new method for detection of compounds which inhibit protein and/or mRNA biosynthesis (e.g. herbicides or antibiotics) comprises expression of a **reporter** gene in vitro in control conditions and in the presence of a test compound, and comparing the results. The method involves incubation of an in vitro transcription-translation mixture containing: an amino acid mixture; plasmid DNA encoding a **reporter** enzyme; an S30 extract from a prokaryote lacking functional **reporter** enzyme activity; a low-mol.wt. compound mixture containing reagents required for protein biosynthesis in vitro; and magnesium ions. The **reporter** gene is preferably a beta-galactosidase (EC-3.2.1.23) lacZ gene, and the S30 extract is from lacZ-negative Escherichia coli MC1061. Detection is by colorimetry or luminescence. The E. coli extract may be supplemented with tRNA and cofactors. The method provides a non-radiolabel assay for **high-throughput** herbicide and drug screening. (18pp)

L4 ANSWER 9 OF 12 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1995-14278 BIOTECHDS
TITLE: Identification of compounds that act on the endothelin receptor; endothelin receptor-agonist and endothelin receptor-antagonist drug screening by luciferase **reporter** gene expression in endothelin receptor-expressing CHO-K1 cell culture
AUTHOR: Kroeger B; Bialojan S; Bollschweiler C
PATENT ASSIGNEE: BASF
PATENT INFO: DE 4408690 21 Sep 1995
APPLICATION INFO: DE 1994-4408690 15 Mar 1994
PRIORITY INFO: DE 1994-4408690 15 Mar 1994
DOCUMENT TYPE: Patent
LANGUAGE: German
OTHER SOURCE: WPI: 1995-329114 [43]

AN 1995-14278 BIOTECHDS
AB A substance which acts on an endothelin receptor (ETR) may be identified by: selecting a cell line that endogenously expresses an ETR, or preparing a cell line that stably expresses a recombinant ETR; transforming the cells with a recombinant vector with a promoter activated by an increase in intracellular second messenger concentration, and a **reporter** gene functionally linked to the promoter; establishing cells with transient expression or stable integration of the vector; exposing selected cells to the test compound; and measuring **reporter** gene expression. Preferably, the **reporter** gene encodes luciferase, and the host cells are CHO-K1 (ATCC CCL 61) cells. Since endothelin is involved in regulation of blood pressure, ligands are potentially useful as hypotensive (ETR-antagonist in smooth muscle cells) or vasodilator (ETR-agonist in endothelium cells) drugs. Both agonists and antagonists may be identified by this method. The method is direct, very sensitivity and permits **high throughput** for mass screening. (7pp)

L4 ANSWER 10 OF 12 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1996-12861 BIOTECHDS
TITLE: Quantitative RT-PCR using closed tube multicolor fluorescence ;
reverse transcription-polymerase chain reaction optimization for e.g. cystic fibrosis transmembrane conductance regulator mRNA expression determination (conference abstract)
AUTHOR: Gibson U; Heid C; Lung A; Stevens J; Williams P M
CORPORATE SOURCE: Genentech; Perkin-Elmer; Appl.Biosyst.
LOCATION: Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080-4990, USA.
SOURCE: Clin.Chem.; (1995) 41, 11, 1686
CODEN: CLCHAU
ISSN: 0009-9147
Nucleic Acids: a Decade of Discovery, San Diego, CA, 16-18 November, 1995.
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1996-12861 BIOTECHDS
AB A novel approach was developed to quantitative reverse transcription-polymerase chain reaction (RT-PCR) using a TaqMan system. Cystic fibrosis transmembrane conductance regulator (CFTR) target mRNA was reverse transcribed and amplified. A DNA probe was designed to bind to a sequence between the PCR primers. This probe contained a **reporter** fluor (FAM) on the 5'-base and a quencher fluor (TAMRA) on the 3'-base. 5' To 3' nuclease activity of Tth DNA-polymerase (EC-2.7.7.7) cleaved the probe during the extension phase of PCR. Cleavage led to increased FAM fluorescence emission. The relative increase in emission was monitored during PCR using a thermal cycler. An internal control template was also designed, which contained the same primer sequence as the CFTR amplicon but was a scrambled internal sequence. An internal control DNA probe was designed to hybridize to the internal control amplicon. The probe contained a different **reporter** fluor (TET), allowing simultaneous detection of both reporters in a single reaction tube. This method provides a convenient and **high throughput** format for quantitative RT-PCR. (0 ref)

L4 ANSWER 11 OF 12 BIOBUSINESS COPYRIGHT 2000 BIOSISDUPLICATE 2
ACCESSION NUMBER: 94:76088 BIOBUSINESS
DOCUMENT NUMBER: 0656290
TITLE: A simple and sensitive **high-throughput** assay for steroid agonists and antagonists.
AUTHOR: White J H; McCuaig K A; Mader S
CORPORATE SOURCE: Dep. Biochem., McIntyre Med. Sci. Bldg., McGill Univ., 3655

[redacted]mmond St., Montreal, Que., H3G [redacted]6, Canada.
SOURCE: [redacted]-Technology (New York), (1994) [redacted]1.12, No.10, Oct., P.1003-1007.
ISSN: 0733-222X.
FILE SEGMENT: UNIQUE
LANGUAGE: ENGLISH
AB We have developed a simple and highly sensitive tissue culture-based assay for the biological activity of steroids and synthetic steroidal compounds.

A DNA cassette, containing a synthetic steroid-inducible promoter controlling the expression of a bacterial chloramphenicol acetyltransferase gene (GRE5-CAT), was inserted into an Epstein-Barry virus (EBV) episomal vector which replicates autonomously in primate and human cells. We then used this promoter/**reporter** system to generate two stably transfected human cell lines. In the cervical carcinoma cell line HeLa, which expresses high levels of glucocorticoid receptor, the GRE5 promoter is inducible over 100-fold by the synthetic glucocorticoid dexamethasone. In the breast carcinoma cell line T47D, which expresses progesterone and androgen receptors, the GRE5 promoter is inducible over 100-fold by either progesterone or dihydrotestosterone. In both cell lines basal expression of CAT activity is strictly dependent on the presence of steroid, so that very low levels of induction can be detected. Thus, the cell lines can be used to test for low levels of agonist activity in steroid antagonists.

L4 ANSWER 12 OF 12 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1995-00281 BIOTECHDS
TITLE: Optimized gene transfer into cystic fibrosis airway cells using novel and improved cationic lipids; lipofection using a cationic lipid-DNA complex and cystic fibrosis transmembrane conductance regulator, for potential gene therapy (conference abstract)
AUTHOR: Cheng S H; Marshall J; Lee E; McNeilly D; Nietupski J; Wysokenski D; Sacks C; Strohm J; Goodrick J; Wang K; Wan N; Yew N; Jiang C; Christopher B; Tsai Y J; Felgner P L; St George J A; Harris D; Smith A E
CORPORATE SOURCE: Genzyme; Vical
LOCATION: Genzyme Corp., One Mountain Road, Framingham, MA 01701, USA.
SOURCE: Gene Ther.Meet.Cold Spring Harbor; (1994) 35
CODEN: 9999M
Gene Therapy, Cold Spring Harbor, New York, 21-25 September, 1994.
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1995-00281 BIOTECHDS
AB To evaluate the transfection activity of cationic lipid:DNA complexes, a **high throughput**, 96 well-based in vitro cytofection assay using beta-galactosidase (EC-3.2.1.23) as **reporter** gene was used for the treatment of cystic fibrosis (CF). Using the optimized conditions, in vitro transfection efficiencies of 60-80% of an immortalized CF airway cell line (CFT-1) were achieved. Transfection of CFT-1 cells using a cystic fibrosis transmembrane conductance regulator-encoding plasmid restored cAMP-stimulated chloride channel activity. Lipid:DNA complexes containing the chloramphenicol-acetyltransferase (CAT, EC-2.3.1.28) **reporter** gene were instilled transtracheally into the lungs of BALB/c mice. CAT activities of up to 2 ng CAT/200 mg tissue were detected following transfection with optimized lipid:DNA formulations. Optimal transfection in vivo was critically dependent on the concentration and the ratio of lipid:DNA used. These results suggest that with further optimization, cationic lipid-mediated gene transfer may be clinically efficacious for gene therapy of cystic fibrosis. (0 ref)

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=> s high throughput

L5 3206 HIGH THROUGHPUT

=> s (signal to noise)

L6 9713 (SIGNAL TO NOISE)

=> s l5 and l6

L7 19 L5 AND L6

=> dup rem l7

PROCESSING COMPLETED FOR L7
L8 12 DUP REM L7 (7 DUPLICATES REMOVED)

=> d ibib abs 1-12

L8 ANSWER 1 OF 12 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1999331852 MEDLINE
DOCUMENT NUMBER: 99331852
TITLE: Photon-burst analysis in two-photon fluorescence excitation
flow cytometry.
AUTHOR: Hanninen P E; Soini J T; Soini E
CORPORATE SOURCE: Department of Medical Physics and Chemistry, University of Turku, Finland.. pekkaha@utu.fi
SOURCE: CYTOMETRY, (1999 Jul 1) 36 (3) 183-8.
Journal code: D92. ISSN: 0196-4763.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199911
ENTRY WEEK: 19991101
AB We studied the use of a dramatically reduced testing zone in combination with two-photon excitation and photon-burst analysis in **high-throughput** rare-event detection simulation using a modified flow cytometer. Two-photon excitation measurements were performed with a mode-locked titanium:sapphire laser. Fluorescence emission was measured with a photon-counting avalanche photodiode. Measured signal was analysed offline by autocorrelation and burst detection methods. Test samples were composed of full blood and orange fluorescent polystyrene nanospheres mixed in full blood. Results show that two-photon fluorescence excitation and time-correlation analysis provide a good **signal**-to-**noise** ratio for rare-event particle detection in a turbid sample environment.

L8 ANSWER 2 OF 12 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1999196682 MEDLINE
DOCUMENT NUMBER: 99196682
TITLE: Viral protease assay based on GAL4 inactivation is applicable to **high-throughput** screening in mammalian cells.
AUTHOR: Lawler J F Jr; Snyder S H
CORPORATE SOURCE: Departments of Neuroscience, Pharmacology and Molecular

iences, and Psychiatry, Johns H[redacted]kins University School Medicine, 725 N., Wolfe Street[redacted]Baltimore, Maryland 21205-2185, USA.
CONTRACT NUMBER: MH-18501 (NIMH)
DA-00674 (NIDA)
GM-07309 (NIGMS)
SOURCE: ANALYTICAL BIOCHEMISTRY, (1999 Apr 10) 269 (1) 133-8.
Journal code: 4NK. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199908
AB We present an assay for viral proteases that relies on the proteolytic cleavage of substrate leading to the dissociation of the yeast transcription factor GAL4. A consensus substrate for the cytomegalovirus protease is fused between the DNA binding and transactivating domains of GAL4. Proteolysis inactivates the transcription factor which drives a luciferase reporter system. The assay is performed in mammalian cells, has a robust **signal**-to-**noise** ratio, and assesses proteolysis in a physiologic context. A unique feature of the assay is its ability to detect inhibitors of viral replication that act on viral targets other than the protease. Copyright 1999 Academic Press.

L8 ANSWER 3 OF 12 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000090196 MEDLINE
DOCUMENT NUMBER: 20090196
TITLE: DNA sequencing using 96-capillary array electrophoresis.
AUTHOR: Pang H; Pavski V; Yeung E S
CORPORATE SOURCE: Ames Laboratory-USDOE and Department of Chemistry, Iowa State University, 50011, USA.
CONTRACT NUMBER: HG-01385 (NHGRI)
SOURCE: JOURNAL OF BIOCHEMICAL AND BIOPHYSICAL METHODS, (1999 Nov 30) 41 (2-3) 121-32.
Journal code: H94. ISSN: 0165-022X.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY WEEK: 20000305
AB Practical DNA sequencing in a rugged capillary array electrophoresis system coupled directly to 96-well microtiter plates is demonstrated. A CCD detector was used to monitor all capillaries simultaneously with laser-induced fluorescence at 1.75 frames per second. The reconstructed electropherograms show good **signal**-to-**noise** ratios and resolution for the entire capillary array. The system used standard dye labeling and image splitting to obtain fluorescence intensities in two wavelength regions to allow calling up to 410 bases for the DNA sequence. The use of a replaceable poly(ethylene oxide) matrix and a protective poly(vinylpyrrolidone) coating allows high separation speed and short turnaround time for **high throughput** DNA sequencing.
Critical evaluation of the system performance over repeated runs with base calling is presented.

L8 ANSWER 4 OF 12 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1999:281320 BIOSIS
DOCUMENT NUMBER: PREV199900281320
TITLE: A fluorescence-based **high throughput** screen for the transporter associated with antigen processing.
AUTHOR(S): Blevitt, Jonathan M.; Fruh, Klaus; Glass, Charlie; Jackson, Michael R.; Peterson, Per A.; Huang, Shaoming (1)
CORPORATE SOURCE: (1) R.W. Johnson Pharmaceutical Research Institute, 3535 General Atomics Court, Suite 100, San Diego, CA, 92121 USA

SOURCE: Journal of Biomolecular Screening, (April, 1999) Vol. 4, No. 2, pp. 87-91.
ISSN: 1087-0571.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The transporter associated with antigen processing (TAP) is essential for antigen presentation by major histocompatibility complex (MHC) class I molecules. Traditional methods used to analyze peptide transport mediated by TAP require radioactive labeling of peptides and time-consuming manipulation of Concanavalin A-Sepharose. Drug discovery research requires rapid and reliable evaluation of large number of samples for bioactivity. To meet these requirements a nonradioactive, HTS assay for peptide transport activity of TAP has been developed. The radioactive label in the traditional assays has been replaced by a fluorescent label without compromising the transport efficiency of labeled peptide or the sensitivity of the assay. The use of multiscreen filtration plates has facilitated higher throughput and eliminated the centrifugation steps used in traditional TAP assays. The HTS assay shows similar kinetic characteristics as compared to the traditional assay. The HTS assay has been adapted on a Quadra(R) 96-320 96-channel pipetting station (Tomtec, Hamden, CT) by optimizing time course, dose response of TAP to peptides and adenosine triphosphate (ATP), **signal/noise** ratio, reproducibility, and reagent stability. This HTS system has been utilized to screen a multiplexed compound library with a maximum of throughput 17,600 compounds per week.

L8 ANSWER 5 OF 12 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1999:281318 BIOSIS
DOCUMENT NUMBER: PREV199900281318
TITLE: A simple statistical parameter for use in evaluation and validation of **high throughput** screening assays.
AUTHOR(S): Zhang, Ji-Hu (1); Chung, Thomas D. Y.; Oldenburg, Kevin R.
CORPORATE SOURCE: (1) Experimental station, E400/5416, DuPont Pharmaceuticals Company, Rt. 141 and Henry Clay Rd., Wilmington, DE, 19880 USA
SOURCE: Journal of Biomolecular Screening, (April, 1999) Vol. 4, No. 2, pp. 67-73.
ISSN: 1087-0571.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The ability to identify active compounds ("hits") from large chemical libraries accurately and rapidly has been the ultimate goal in developing **high-throughput** screening (HTS) assays. The ability to identify hits from a particular HTS assay depends largely on the suitability or quality of the assay used in the screening. The criteria or parameters for evaluating the "suitability" of an HTS assay for hit identification are not well defined and hence it still remains difficult to compare the quality of assays directly. In this report, a screening window coefficient, called "Z-factor," is defined. This coefficient is reflective of both the assay signal dynamic range and the data variation associated with the signal measurements, and therefore is suitable for assay quality assessment. The Z-factor is a dimensionless, simple statistical characteristic for each HTS assay. The Z-factor provides a useful tool for comparison and evaluation of the quality of assays, and can be utilized in assayoptimization and validation.

L8 ANSWER 6 OF 12 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 4
ACCESSION NUMBER: 1999:180268 BIOSIS
DOCUMENT NUMBER: PREV199900180268
TITLE: **High throughput** scintillation proximity assay for the identification of FKBP-12 ligands.

AUTHOR(S): [redacted]ziani, Francesca; Aldegheri, L[redacted]a; Terstappen, Georg C.
(1)
CORPORATE SOURCE: (1) Department of Lead Discovery, GlaxoWellcome Medicines Research Centre, Via A. Fleming 4, 37135, Verona Italy
SOURCE: Journal of Biomolecular Screening, (Feb., 1999) Vol. 4, No.
1, pp. 3-7.
ISSN: 1087-0571.
DOCUMENT TYPE: Article
LANGUAGE: English

AB A **high throughput** scintillation proximity assay (SPA) was developed to identify novel ligands of FKBP-12, an immunophilin with peptidyl prolyl isomerase (rotamase) activity. Recombinant histidine-tagged FKBP-12 was ex-pressed in Escherichia coli, purified by metal ion affinity chromatography, and immobilized to SPA beads by an antibody that recognizes the histidine tag of the recombinant protein. Using 1 nM (3H) FK506, a well-known macrolid ligand of FKBP-12, specific binding was saturable and accounted for 95% of total binding. Analysis of saturation and homologous displacement isotherms indicated the existence of a single binding site with a KD value of 1.6 nM. The specificity of (3H) FK506 binding was demonstrated in displacement experiments and showed that rapamycin, another macrolid, was as active as FK506 (IC50 of 3.5 and 3.2 nM, respectively), whereas GPI-1046, a prototype of small molecular compounds with neurotrophic properties and affinity for FKBP-type immunophilins, was more than 1000-fold less active. The high **signal**-to-**noise** ratio of 30, together with small standard deviations, makes this novel assay well suited for automated **high throughput** screening.

L8 ANSWER 7 OF 12 MEDLINE
ACCESSION NUMBER: 1999000922 MEDLINE
DOCUMENT NUMBER: 99000922
TITLE: Routine DNA sequencing of 1000 bases in less than one hour by capillary electrophoresis with replaceable linear polyacrylamide solutions.
AUTHOR: Salas-Solano O; Carrilho E; Kotler L; Miller A W; Goetzinger W; Sosic Z; Karger B L
CORPORATE SOURCE: Barnett Institute, Northeastern University, Boston, Massachusetts 02115, USA.
SOURCE: ANALYTICAL CHEMISTRY, (1998 Oct 1) 70 (19) 3996-4003. Journal code: 4NR. ISSN: 0003-2700.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
ENTRY MONTH: 199901
ENTRY WEEK: 19990104

AB Long, accurate reads are an important factor for **high-throughput** de novo DNA sequencing. In previous work from this laboratory, a separation matrix of high-weight-average molecular mass (HMM) linear polyacrylamide (LPA) at a concentration of 2% (w/w) was used to separate 1000 bases of DNA sequence in 80 min with an accuracy close to 97% (Carrilho, E.; et al. Anal. Chem. 1996, 68, 3305-3313). In the present work, significantly improved speed and sequencing accuracy have been achieved by further optimization of factors affecting electrophoretic separation and data processing. A replaceable matrix containing a mixture of 2.0% (w/w) HMM (9 MDa) and 0.5% (w/w) low-weight-average molecular mass (50 kDa) LPA was employed to enhance the separation of DNA sequencing fragments in CE. Experimental conditions, such as electric field strength and column temperature, as well as internal diameter of the capillary column, have been optimized for this mixed separation matrix. Under these conditions, in combination with energy-transfer (BigDye) dye-labeled primers for high **signal**-to-**noise** ratio and a newly developed expert system for base calling, the electrophoretic separation of 1000 DNA sequencing fragments of both standard (M13mp18) and cloned

single-stranded templates from human chromosome could be routinely achieved in less than 55 min, with a base-calling accuracy between 98 and 99%. Identical read length, accuracy, and migration time were achieved in more than 300 consecutive runs in a single column.

L8 ANSWER 8 OF 12 MEDLINE
ACCESSION NUMBER: 1998271777 MEDLINE
DOCUMENT NUMBER: 98271777
TITLE: Solid-state DNA sizing by atomic force microscopy.
AUTHOR: Fang Y; Spisz T S; Wiltshire T; D'Costa N P; Bankman I N; Reeves R H; Hoh J H
CORPORATE SOURCE: Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.
CONTRACT NUMBER: HG01518-02
HG0095-07
SOURCE: ANALYTICAL CHEMISTRY, (1998 May 15) 70 (10) 2123-9.
Journal code: 4NR. ISSN: 0003-2700.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
ENTRY MONTH: 199809
ENTRY WEEK: 19980904
AB Atomic force microscopy (AFM) allows rapid, accurate, and reproducible visualization of DNA adsorbed onto solid supports. The images reflect the lengths of the DNA molecules in the sample. Here we propose a solid-state DNA sizing (SSDS) method based on AFM as an analytical method for **high-throughput** applications such as finger-printing, restriction mapping, +/- screening, and genotyping. For this process, the sample is first deposited onto a solid support by adsorption from solution. It is then dried and imaged under ambient conditions by AFM. The resulting images are subjected to automated determination of the lengths of the DNA molecules on the surface. The result is a histogram of sizes that is similar to densitometric scans of DNA samples separated on gels. A direct comparison of SSDS with agarose gel electrophoresis for +/- screening shows that it produces equivalent results. Advantages of SSDS include reduced sample size (i.e., lower reagent costs), rapid analysis of single samples, and potential for full automation using available technology. The high sensitivity of the method also allows the number of polymerase chain reaction cycles to be reduced to 15 or less. Because the high **signal**-to-**noise** ratio of the AFM allows for direct visualization of DNA-binding proteins, different DNA conformations, restriction enzymes, and other DNA modifications, there is potential for dramatically improving the information content in this type of analysis.

L8 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1998:342418 BIOSIS
DOCUMENT NUMBER: PREV199800342418
TITLE: **High-throughput** large-aperture prism prefilter for ultraviolet resonance Raman spectroscopy.
AUTHOR(S): Kaminaka, Shoji; Mathies, Richard A. (1)
CORPORATE SOURCE: (1) Dep. Chemistry, Univ. Calif., Berkeley, CA 94720 USA
SOURCE: Applied Spectroscopy, (March, 1998) Vol. 52, No. 3, pp. 469-473.
ISSN: 0003-7028.
DOCUMENT TYPE: Article
LANGUAGE: English
AB For the acquisition of high-quality ultraviolet resonance Raman spectra of strongly scattering samples such as membrane protein suspensions, an f/3.5 Littrow prism prefilter has been designed, built, and characterized. This prefilter has a Czerny-Turner configuration, and its focal length is 25 cm. The apex angle of the dispersive prism (20degree) was chosen to provide maximum performance in the 220 to 240 nm range. The prism prefilter significantly reduced stray background due to Rayleigh

scattering and [redacted]ible fluorescence, while maint[redacted]ing a low dispersion of
1300 cm-1/mm at 253 nm as well as a large f/3.5 aperture. The sharpness of
the transmission edge (at 242 nm, the T = 0% to 95% transition occurs in 1.3 nm) quantitates its effectiveness as a sharp-cut Rayleigh scattering filter. The total throughput of the prefilter is apprx60% at 235 nm and apprx50% at 632.8 nm. The utility of this prefilter is demonstrated by obtaining high **signal**-to-**noise** resonance Raman spectra of bacteriorhodopsin in a purple membrane suspension with 239.5 nm excitation.

L8 ANSWER 10 OF 12 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1999:82963 BIOSIS
DOCUMENT NUMBER: PREV199900082963
TITLE: Development of nonseparation binding and functional assays for G protein-coupled receptors for **high throughput** screening: Pharmacological characterization of the immobilized CCR5 receptor on flashplate.
AUTHOR(S): Bosse, Roger; Garlick, Russell; Brown, Beverly; Menard, Luc
(1)
CORPORATE SOURCE: (1) BioSignal Inc., 1744 William Street, Montreal, PQ H3J Canada
SOURCE: Journal of Biomolecular Screening, (Winter, 1998) Vol. 3, No. 4, pp. 285-292.
ISSN: 1087-0571.
DOCUMENT TYPE: Article
LANGUAGE: English
AB G protein-coupled receptors (GPCRs) represent a very important class of drug targets. The development of microformatted nonseparation assays constitute a key step in the process of assay development for **high throughput** drug screening (HTS). We have developed a microformatted nonseparation assay for membrane preparations containing the CCR5 GPCR using FlashPlate(R) microplates (Packard Instrument Company, Meriden, CT). The pharmacodynamic (radioligand-binding) and functional (agonist-stimulated (35S)GTPgammaS binding) properties of this receptor observed in FlashPlate-based assays were compared with standard filtration assays. Saturation binding experiments performed using either assay platform revealed identical Kd for (125I)-MIP-1 beta (0.7 nM). Comparable **signal**-to-**noise** ratios (SNR), similar affinities (Ki), and identical order of potency (RANTES apprxeq MIP-1beta > MIP-1alpha) were observed following competition binding assays in both platforms. In functional assays, the order of potency for different agonists were similar in both platforms with RANTES apprxeq MIP-1beta gtoreq MIP-1alpha, which correspond to the relative affinities determined for the three ligands in competition binding experiments. Because similar pharmacologic properties were obtained in both FlashPlate microplates and standard filtration platforms, we conclude that FlashPlate microplates could provide a valuable nonseparation platform for primary and secondary HTS for this and possibly other GPCRs.

L8 ANSWER 11 OF 12 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 89270998 MEDLINE
DOCUMENT NUMBER: 89270998
TITLE: Fluorescence-based viability assay for studies of reactive drug intermediates.
AUTHOR: Leeder J S; Dosch H M; Harper P A; Lam P; Spielberg S P
CORPORATE SOURCE: Division of Clinical Pharmacology/Toxicology, Hospital for Sick Children, Toronto, Ontario, Canada.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1989 Mar) 177 (2) 364-72.
Journal code: 4NK. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: [redacted]ority Journals
ENTRY MONTH: [redacted]909

AB Studies of drug toxicity, toxicologic structure-function relationships, screening of idiosyncratic drug reactions, and a variety of cytotoxic events and cellular functions in immunology and cell biology require the sensitive and rapid processing of often large numbers of cell samples. This report describes the development of a high-sensitivity, **high-throughput** viability assay based on (a) the carboxyfluorescein derivative 2'-7'-biscarboxyethyl-5(6)-carboxyfluorescein (BCECF) as a vital dye, (b) instrumentation capable of processing multiple small (less than 100 cells) samples, and (c) a 96-well unidirectional vacuum filtration plate. Double staining of cultured peripheral blood mononuclear cells with BCECF and propidium iodide (PI) showed no overlap between PI+ (nonviable) and BCECF+ (viable) cells by flow cytometric analysis. Optimal conditions were developed for dye loading and minimizing physical cell damage and fluorescence quench during the assay procedure. The ratio of BCECF fluorescence to internal standard fluorescent particles was linear from 40 to greater than 20,000 cells with a **signal:noise** ratio of approximately 3 at 40 cells/well. Sulfamethoxazole hydroxylamine (SMX-HA) was used as a model toxic drug metabolite to explore the validity of the BCECF procedure. SMX-HA, but not its parent compound sulfamethoxazole, resulted in a dose dependent loss of cellular fluorescence and the parallel accumulation of PI+ nonviable cells. When compared to the currently used tetrazolium dye reduction viability assay, the BCECF method was 3-fold more sensitive, greater than 10-fold faster, and required 1/10-1/100 the cell numbers.

L8 ANSWER 12 OF 12 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1981:160658 BIOSIS
DOCUMENT NUMBER: BA71:30650
TITLE: AN ASSAY FOR PICOMOLE OF TYROSINE AND RELATED PHENOLS AND ITS APPLICATION TO THE MEASUREMENT OF PHENYL ALANINE HYDROXYLASE ACTIVITY.
AUTHOR(S): BAILEY S W; AYLING J E
CORPORATE SOURCE: UNIV. TEX., SAN ANTONIO, TEX. 78285.
SOURCE: ANAL BIOCHEM, (1980) 107 (1), 156-164.
CODEN: ANBCA2. ISSN: 0003-2697.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Detection of tyrosine and related compounds by their native fluorescence is coupled to high-pressure liquid chromatography to provide a highly specific assay linear over almost 5 orders of magnitude, with a limit of sensitivity of 2 pmol (**signal/noise** = 2). Requiring no derivatization and minimal sample preparation, **high throughput** is obtained. The technique is illustrated by its application to the quantitation of tyrosine produced by phenylalanine hydroxylase, and tyrosine levels in plasma and tissue extracts. Following removal of protein by precipitation with trichloroacetic acid and centrifugation, 30 samples/h can be chromatographed. The maximum volume which can be injected onto a 10 .times. 0.46-cm column of 5-.mu.m C18 bonded phase silica, without peak distortion, is 50 .mu.l, corresponding to a minimum detectable concentration of 4 .times. 10-8 M. Optimization of the fluorometric and chromatographic parameters allows analysis of other hydroxylated aromatic compounds, as exemplified by the separation of o-, m- and p-tyrosine, and dihydroxyphenylalanine.

=> d his

(FILE 'HOME' ENTERED AT 11:25:03 ON 14 MAY 2000)

FILE 'BIOBUSINESS, LIFESCI, CAPLUS' ENTERED AT 11:27:47 ON 14 MAY 2000

FILE 'BIOBUSINESS, BIOTECHDS' ENTERED AT 11:32:12 ON 14 MAY 2000

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L1          224 S HI THROUGHPUT
L2         4290 S RE TER
L3           14 S L1 AND L2
L4           12 DUP REM L3 (2 DUPLICATES REMOVED)

     FILE 'MEDLINE, BIOSIS, BIOTECHNO' ENTERED AT 11:44:03 ON 14 MAY 2000
L5         3206 S HIGH THROUGHPUT
L6         9713 S (SIGNAL TO NOISE)
L7           19 S L5 AND L6
L8           12 DUP REM L7 (7 DUPLICATES REMOVED)

=> s reporter

L9        50715 REPORTER

=> s l2 or l9

L10       50715 L2 OR L9

=> s l9 and l6

L11          39 L9 AND L6

=> dup rem l11

PROCESSING COMPLETED FOR L11
L12          18 DUP REM L11 (21 DUPLICATES REMOVED)

=> s l12 not l8

L13          17 L12 NOT L8

=> d ibib abs 1-17


L13  ANSWER 1 OF 17  MEDLINE
ACCESSION NUMBER:    2000210399     MEDLINE
DOCUMENT NUMBER:     20210399
TITLE:               Comparison of formats for the development of fiber-optic
                     biosensors utilizing sol-gel derived materials entrapping
                     fluorescently-labelled protein.
AUTHOR:              Flora K; Brennan J D
CORPORATE SOURCE:    Department of Chemistry, McMaster University, Hamilton,
                     Ontario, Canada.
SOURCE:              ANALYST, (1999 Oct) 124 (10) 1455-62.
                     Journal code: 4OS. ISSN: 0003-2654.
PUB. COUNTRY:        ENGLAND: United Kingdom
                     Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
ENTRY MONTH:         200006
ENTRY WEEK:          20000603
AB   The development of fiber-optic biosensors requires that a biorecognition
     element and a fluorescent reporter group be immobilized at or
     near the surface of an optical element such as a planar waveguide or
     optical fiber. In this study, we examined a model biorecognition element-
     reporter group couple consisting of human serum albumin that was
     site-selectively labelled at Cys 34 with iodoacetoxy-nitrobenzoxadiazole
     (HSA-NBD). The labelled protein was encapsulated into sol-gel derived
     materials that were prepared either as monoliths, as beads that were
     formed at the distal tip of a fused silica optical fiber, or as thin
films
     that were dipcast along the length of a glass slide or optical fiber. For
     fiber-based studies, the entrapped protein was excited using a
     helium-cadmium laser that was launched into a single optical fiber, and
     emission was separated from the incident radiation using a perforated
     mirror beam-splitter, and detected using a monochromator-photomultiplier
     tube assembly. Changes in fluorescence intensity were generated by
     denaturant-induced conformational changes in the protein or by iodide
     quenching. The analytical parameters of merit for the different
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encapsulation formats, including minimum protein loading level, response time and limit-of-detection, were examined, as were factors such as protein accessibility, leaching and photobleaching. Overall, the results indicated that both beads and films were suitable for biosensor development. In both formats, a substantial fraction of the entrapped protein remained accessible, and the entrapped protein retained a large degree of conformational flexibility. Thin films showed the most rapid response times, and provided good detection limits for a model analyte. However, the entrapment of proteins into beads at the distal tip of fibers provided better **signal**-to-**noise** and signal-to-background ratios, and required less protein for preparation. Hence, beads appear to be the most viable method for interfacing of proteins to optical fibers.

L13 ANSWER 2 OF 17 MEDLINE
ACCESSION NUMBER: 2000054873 MEDLINE
DOCUMENT NUMBER: 20054873
TITLE: cobA, a red fluorescent transcriptional **reporter** for Escherichia coli, yeast, and mammalian cells.
AUTHOR: Wildt S; Deuschle U
CORPORATE SOURCE: Pharma Division, Preclinical CNS Research and GeneTechnologies, Molecular Neurobiology, F. Hoffmann-La Roche AG, Basel, Switzerland.
SOURCE: NATURE BIOTECHNOLOGY, (1999 Dec) 17 (12) 1175-8. Journal code: CQ3. ISSN: 1087-0156.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY WEEK: 20000303
AB We demonstrate the use of Propionibacterium freudenreichii uroporphyrinogen III methyltransferase (cobA) as a **reporter** of gene expression in Escherichia coli, fission yeast, and mammalian cells. Overexpression of cobA in cells resulted in bright red fluorescence that was visualized with standard fluorescence microscopy and fluorescence-activated cell sorting analysis at the single-cell level. As with green fluorescent protein (GFP), no addition of exogenous substrate was required. When expressed in Chinese hamster ovary cells from a bicistronic transcript, cobA and GFP gave rise to fluorescence signals of similar intensity. The bright red fluorescence generated by the cobA **reporter** promises a better **signal**-to-**noise** ratio than blue and green fluorescent **reporter** systems, as autofluorescence and light scattering of cells, media, and materials are reduced in the red wavelengths.

L13 ANSWER 3 OF 17 MEDLINE
ACCESSION NUMBER: 2000011244 MEDLINE
DOCUMENT NUMBER: 20011244
TITLE: Stress responses as a tool To detect and characterize the mode of action of antibacterial agents.
AUTHOR: Bianchi A A; Baneyx F
CORPORATE SOURCE: Department of Bioengineering, University of Washington, Seattle, Washington 98195, USA.
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1999 Nov) 65 (11) 5023-7. Journal code: 6K6. ISSN: 0099-2240.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY WEEK: 20000204
AB Single-copy gene fusions between the lacZ **reporter** gene and Escherichia coli strains containing promoters induced by cold shock (cspA), cytoplasmic stress (ibp), or protein misfolding in the cell envelope (P3rpoH) were constructed and tested to determine their ability to detect antibacterial agents while simultaneously providing information

on their cellul[redacted]targets. Antibiotics that affe[redacted]prokaryotic ribosomes selectively ind[redacted]ed the cspA::lacZ or ibp::lacZ g[redacted]e fusion, depending on their mode of action. The membrane-damaging peptide polymyxin B induced both the P3rpoH::lacZ and ibp::lacZ fusions, while the beta-lactam antibacterial agent carbenicillin activated only the P3rpoH promoter. Nalidixic acid, a compound that causes DNA damage, downregulated beta-galactosidase synthesis from P3rpoH but had little effect on expression of the **reporter** enzyme from either the cspA or ibp promoter. All model antibiotics could be identified over a wide range of sublethal concentrations with **signal**-to-**noise** ratios between 2 and 11. A blue halo assay was developed to rapidly characterize the modes of action of antibacterial agents by visual inspection, and this assay was used to detect chloramphenicol secreted into the growth medium of Streptomyces venezuelae cultures. This simple system holds promise for screening natural or combinatorial libraries of antimicrobial compounds.

L13 ANSWER 4 OF 17 MEDLINE
ACCESSION NUMBER: 1999045819 MEDLINE
DOCUMENT NUMBER: 99045819
TITLE: Development of a green fluorescent protein **reporter** for a yeast genotoxicity biosensor.
AUTHOR: Billinton N; Barker M G; Michel C E; Knight A W; Heyer W D;
Goddard N J; Fielden P R; Walmsley R M
CORPORATE SOURCE: Department of Biomolecular Sciences, UMIST, Manchester, UK.
SOURCE: BIOSENSORS AND BIOELECTRONICS, (1998 Oct 1) 13 (7-8) 831-8.
Journal code: AKA. ISSN: 0956-5663.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY WEEK: 19990304
AB A **reporter** system, constructed for a laboratory screen for new genes involved in DNA repair in the brewer's yeast Saccharomyces cerevisiae, has been developed for use in a genotoxicity biosensor. The strain produces green fluorescent protein (yEGFP) when DNA damage has occurred. yEGFP is codon optimised for yeasts. The **reporter** does not respond to chemicals which delay mitosis, and responds appropriately to the genetic regulation of DNA repair. Data is presented which demonstrate strain improvements appropriate to biosensor technology: improved **signal** to **noise** ratio, ease of data collection and uncomplicated material handling.

L13 ANSWER 5 OF 17 MEDLINE
ACCESSION NUMBER: 1998447473 MEDLINE
DOCUMENT NUMBER: 98447473
TITLE: A highly sensitive and specific assay using a novel human growth hormone cDNA **reporter** gene regulated by the human interleukin-4 inducible germline epsilon transcript promoter.
AUTHOR: Jenh C H; Cox M A; Lundell D; Narula S K; Zavodny P J
CORPORATE SOURCE: Department of Immunology, Schering-Plough Research Institute, Kenilworth, NJ 07033, USA.. chung-her.jenh@spcorp.com
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1998 Aug 1) 217 (1-2) 87-95.
Journal code: IFE. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199901
ENTRY WEEK: 19990104
AB We have successfully developed a highly sensitive and specific assay system for human interleukin-4 (IL-4) regulated gene expression. It is

based on a huma[redacted]ijoye cell line with the germl[redacted] epsilon transcript promoter joined[redacted] the human growth hormone (hGH[redacted]DNA. The germline epsilon transcript promoter is responsive to IL-4 and involved in immunoglobulin heavy chain class switching. We cloned hGH complementary DNA (cDNA) as the **reporter** gene instead of using conventional hGH genomic DNA which failed to generate any IL-4 inducible clone in human Jijoye cells. The two IL-4 inducible cell lines with the hGH cDNA **reporter** show high **signal/noise** ratio for IL-4-mediated induction (60-90 fold). The response to IL-4 is dose-dependent with ED50 of 10 pM. As expected, there is no response to other human cytokines and growth factors, as well as mouse IL-4. The mutant hIL-4 antagonist hIL-4.Ŷ124D inhibits the induction mediated by native hIL-4. These IL-4 inducible cell lines provide a sensitive, specific assay system to study IL-4-regulated gene expression, and in particular the regulation of the germline epsilon promoter.

L13 ANSWER 6 OF 17 MEDLINE
ACCESSION NUMBER: 1998348965 MEDLINE
DOCUMENT NUMBER: 98348965
TITLE: Comparison of mutant forms of the green fluorescent protein as expression markers in Chinese hamster ovary (CHO) and Saccharomyces cerevisiae cells.
AUTHOR: Natarajan A; Subramanian S; Srienc F
CORPORATE SOURCE: Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, USA.
SOURCE: JOURNAL OF BIOTECHNOLOGY, (1998 Jun 11) 62 (1) 29-45. Journal code: AL6. ISSN: 0168-1656.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY WEEK: 19981004
AB Several green fluorescent protein (Gfp) mutants with increased cellular fluorescence compared to the wildtype protein have recently been generated. We have expressed and compared wildtype Gfp and mutants S65T, F100S/ M154T/V164A, F64L/S65T, and S65A/V68L/S72A under identical growth conditions in CHO and Saccharomyces cerevisiae cells. The results suggest that the last two Gfp mutants are the best candidates as **reporter** proteins, and they provide a high **signal**-to-**noise** ratio in both systems. Single gene copy expression of these mutant forms is easily detectable over background autofluorescence. All Gfps are highly stable within cells, with an estimated 1/2-life between 7 h (wildtype) and 70 h (F100S/M154T/V164A) in S. cerevisiae cells. Although this limits their use in examining rapid cellular events without further modification, Gfp is expected to be a useful marker for monitoring the physiological state of cells in bioreactors using on-line probes.

L13 ANSWER 7 OF 17 MEDLINE
ACCESSION NUMBER: 97459031 MEDLINE
DOCUMENT NUMBER: 97459031
TITLE: Sensitive multicolor fluorescence in situ hybridization using catalyzed **reporter** deposition (CARD) amplification.
AUTHOR: Speel E J; Ramaekers F C; Hopman A H
CORPORATE SOURCE: Department of Molecular Cell Biology & Genetics, University of Maastricht, The Netherlands.
SOURCE: JOURNAL OF HISTOCHEMISTRY AND CYTOCHEMISTRY, (1997 Oct) 45 (10) 1439-46. Journal code: IDZ. ISSN: 0022-1554.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: [redacted]rity Journals; Cancer Journals
ENTRY MONTH: [redacted]12
ENTRY WEEK: 19971204

AB We describe the simultaneous localization of DNA sequences in cell and chromosome preparations by means of differently fluorochrome-labeled (AMCA, FITC, TRITC) tyramides using the catalyzed **reporter** deposition (CARD) procedure. For this purpose, repeated as well as single-copy DNA probes were labeled with biotin, digoxigenin, and FITC, hybridized, and visualized with three different cytochemical detection systems based on horseradish peroxidase conjugates. These were sequentially applied to interphase nuclei and metaphase chromosomes at low concentrations to prevent crossreaction and nonspecific background. In situ localized peroxidase activity was visualized by the deposition of fluorochrome-labeled tyramide molecules. To allow specific deposition of a second and a third tyramide conjugate for multiple-target fluorescence in situ hybridization (FISH), remaining peroxidase activity was always completely inactivated by a mild acid treatment before application of the next peroxidase conjugate. The CARD reactions were optimized for maximal **signal**-to-**noise** ratio and discrete localization by tuning reaction time, H2O2, and tyramide concentrations. For both repeated and single-copy DNA targets, high FISH signal intensities were obtained, providing improvement of sensitivity over conventional indirect detection systems. In addition, the fluorescence CARD detection system proved to be highly efficient and easy to implement in multiple-labeling studies, such as reported here for FISH.

L13 ANSWER 8 OF 17 MEDLINE
ACCESSION NUMBER: 97356268 MEDLINE
DOCUMENT NUMBER: 97356268
TITLE: Quantitative comparison of global carbohydrate structures of glycoproteins using LC-MS and in-source fragmentation.
AUTHOR: Mazsaroff I; Yu W; Kelley B D; Vath J E
CORPORATE SOURCE: Genetics Institute, Inc, Andover, Massachusetts 01810, USA.
SOURCE: ANALYTICAL CHEMISTRY, (1997 Jul 1) 69 (13) 2517-24. Journal code: 4NR. ISSN: 0003-2700.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
ENTRY MONTH: 199710

AB A comparative method for the quantitative analysis of the ratio of oxonium fragment (**reporter**) ions derived from sialic acid and N-acetylhexosamine residues on a large intact glycoprotein, the B domain of recombinant human factor VIII (rhFVIII), was developed. The method utilized liquid chromatography-electrospray ionization mass spectrometry (LC-ESI MS) on a single-quadrupole instrument. During development, systematic approaches such as full-matrix and simplex strategies were used for the optimization of the **signal**-to-**noise** ratio by controlling source temperature and cone voltage. The method was found to be precise (RSD = 0.84%), sensitive (capable of differentiating 1 sialic acid residue change among at least 29 sialic acids on a 103-kDa glycoprotein that is 38% carbohydrate), applicable to a wide range of loading (11.6-372 micrograms of FVIII), and accurate according to a comparison to matrix-assisted laser desorption-ionization time-of-flight mass spectrometry. Combining the method with enzymatic removal of N-glycans, selective O-glycan analysis was also performed leading to differential fragment ion analysis ascribed to N- and O-glycans. Quantitative ESI in-source dissociation MS combined with LC can generally be used for glycoproteins, as one of the indicators, to compare the distribution of carbohydrate residues over N- and O-glycans, to investigate their isoforms, and compare batch-to-batch characteristics of biopharmaceuticals.

L13 ANSWER 9 OF 17 MEDLINE

ACCESSION NUMBER: [redacted]6293 MEDLINE
DOCUMENT NUMBER: [redacted]6293
TITLE: A highly sensitive cytotoxicity assay based on the release of **reporter** enzymes, from stably transfected cell lines.
AUTHOR: Schafer H; Schafer A; Kiderlen A F; Masihi K N; Burger R
CORPORATE SOURCE: Department of Immunology, Robert Koch-Institute, Berlin, Germany.
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1997 May 12) 204 (1) 89-98.
Journal code: IFE. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199709
ENTRY WEEK: 19970904

AB The well-established methods of generating stably transfected cell lines, and the detection of nanomolar amounts of an enzyme in a fast and reproducible assay, were utilised to establish non-radiometric cytotoxicity assays. In these assay systems, the detection of released enzymes was used to quantitate the leakage of intracellular proteins after membrane disintegration. Target cell lines were transfected with a luciferase **reporter** gene under the control of a strong eucaryotic promoter. Release of the intracellular expressed enzyme into the culture supernatant occurred after membrane perforation and was measured as an indicator of cellular death. The quantitation of released enzyme was a reliable indicator of cell death initiated either by complement-mediated killing, or by cell-mediated cytotoxicity. This system was initially established with P815 mastocytoma cells as an example of a target cell line. Transfection with the firefly luciferase gene provided an intracellular enzyme absent in mammalian cells. In a parallel approach, P815 and BW5147 target cells were transfected with bacterial beta-galactosidase to provide a similar cytotoxicity system. This enzyme, however, has a considerably longer half life in tissue culture medium than luciferase. In a direct comparison between the standard 51Cr release and beta-galactosidase release, the enzyme release showed a much higher **signal**-to-**noise** ratio, i.e., low background and high induced release if spontaneous release and detergent induced maximal lysis were measured. Since a wide range of human and murine cell lines can be stably transfected and several **reporter** genes are available, the system should provide an alternative for conventional cytotoxicity assays.
The detection of released enzymes by colorimetric or luminometric methods makes this cytotoxicity assay independent of radionuclides. The sensitivity of luminometric enzyme detection systems should also permit the measurement of apoptotic processes and might make in vivo studies of cellular death using transgenic animals feasible.

L13 ANSWER 10 OF 17 MEDLINE
ACCESSION NUMBER: 97155512 MEDLINE
DOCUMENT NUMBER: 97155512
TITLE: Genetically modified Escherichia coli for colorimetric detection of inorganic and organic Hg compounds.
AUTHOR: Klein J; Altenbuchner J; Mattes R
CORPORATE SOURCE: Institute of Industrial Genetics, University of Stuttgart, Germany.
SOURCE: EXS, (1997) 80 133-51.
Journal code: BFZ.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704

ENTRY WEEK: [redacted]70404
AB A sensitive colorimetric bacterial system was developed for the detection of Hg(II) and organomercury compounds. The bioactive species, a recombinant Escherichia coli, produces proportionally elevated levels of the enzyme beta-galactosidase with increasing amounts of Hg. This is due to a **reporter** plasmid which carriers a Hg(II)-inducible promoter (mer promoter) from the Hg resistance transposon Tn501 regulating the transcription of a promoterless lacZ gene. Additionally, a pMB1 origin of replication without the natural RNA polymerase start site is fused downstream of the mer promoter leading to a Hg(II)-inducible plasmid replication, which results in an improved **signal**-to-**noise** ratio. To enhance the sensitivity of this cellular biosensor, the transport proteins for Hg(II) uptake are constitutively produced by a helper plasmid. To enable the detection of organically bound Hg, the Streptomyces lividans organomercurical lyase, an enzyme which catalyses the cleavage of C-Hg-bonds of organomercurial compounds, is also provided by the helper plasmid. Hg(II) and phenylmercuric acetate (PMA) concentrations as low as 5 x 10(-10) M (0.1 ppb) may be detected within a few minutes.

L13 ANSWER 11 OF 17 MEDLINE
ACCESSION NUMBER: 96323253 MEDLINE
DOCUMENT NUMBER: 96323253
TITLE: Simultaneous fluorescence-activated cell sorter analysis of two distinct transcriptional elements within a single cell using engineered green fluorescent proteins.
AUTHOR: Anderson M T; Tjioe I M; Lorincz M C; Parks D R; Herzenberg L A; Nolan G P; Herzenberg L A
CORPORATE SOURCE: Department of Genetics, Stanford University School of Medicine, CA 94305, USA.
CONTRACT NUMBER: CA 42609 (NCI)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Aug 6) 93 (16) 8508-11. Journal code: PV3. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199611
AB Green fluorescent protein (GFP) is widely used as a **reporter** gene in both prokaryotes and eukaryotes. However, the fluorescence levels of wild-type GFP (wtGFP) are not bright enough for fluorescence-activated cell sorting or flow cytometry. Several GFP variants were generated that are brighter or have altered excitation spectra when expressed in prokaryotic cells. We engineered two GFP genes with different combinations of these mutations, GFP(S65T,V163A) termed GFP-Bex1, and GFP(S202F,T203I,V163A) termed GFP-Vex1. Both show enhanced brightness and improved **signal**-to-**noise** ratios when expressed in mammalian cells and appropriately excited, compared with wtGFP. Each mutant retains only one of the two excitation peaks of the wild-type protein. GFP-Bex1 excites at 488 nm (blue) and GFP-Vex1 excites at 406 nm (violet), both of which are available laser lines. Excitation at these wavelengths allows for the independent analyses of these mutants by fluorescence-activated cell sorting, permitting simultaneous, quantitative detection of expression from two different genes within single mammalian cells.

L13 ANSWER 12 OF 17 MEDLINE
ACCESSION NUMBER: 92254989 MEDLINE
DOCUMENT NUMBER: 92254989
TITLE: Herpes simplex virus thymidine kinase enzymatic assay in transient transfection experiments using thymidine kinase-deficient cells.

AUTHOR: [redacted]sson J J; McIvor R S
CORPORATE SOURCE: [redacted]partment of Laboratory Medicine[redacted]d Pathology, University
of Minnesota, Minneapolis 55455.
CONTRACT NUMBER: AI27416 (NIAID)
SOURCE: ANALYTICAL BIOCHEMISTRY, (1991 Dec) 199 (2) 232-7.
Journal code: 4NK. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199208

AB An enzymatic assay for herpes virus simplex type 1 thymidine kinase (HSV-TK) that was sensitive enough to quantitate intracellular levels of enzyme transiently expressed after transfection of HSV-TK vectors into TK-deficient cells using the DNA-calcium phosphate coprecipitation technique is described. TK activity in extracts of transfected cells was determined by binding of [methyl-3H]thymidylate product to thin layers of polyethyleneimine (PEI)-impregnated cellulose. The assay used high-specific-activity [methyl-3H]thymidine as substrate, which required removal of anionic material on a column of PEI-cellulose to enhance the **signal**-to-**noise** ratio. The assay was linear over a wide range with respect to the amount of HSV-TK plasmid transfected or content of HSV-TK enzyme in cell extracts. To validate the assay in transient expression experiments, HSV-TK and chloramphenicol acetyltransferase (CAT) plasmids were cotransfected into NIH/3T3 tk- fibroblasts. Transient TK and CAT levels were concordant in cell extracts prepared from replicate plates of transfected cells. Normalizing the transient TK activity for CAT activity from the cotransfected "internal standard" CAT plasmid improved precision significantly, reducing the sample-to-sample coefficient of variation from 41 to 19%. CAT normalization reduced experimental variability mostly by correcting outlying results in transfection efficiency. The HSV-TK **reporter** gene system based on TK enzymatic assay was thus subject to experimental variation similar to that of the well-established CAT **reporter** function, demonstrating its utility in transient gene expression analysis.

L13 ANSWER 13 OF 17 MEDLINE
ACCESSION NUMBER: 91270269 MEDLINE
DOCUMENT NUMBER: 91270269
TITLE: A Plasmodium falciparum-specific reverse target capture assay.
AUTHOR: Chen G X; Zhu J D; Plitt J R; Weiler A K; Zolg J W
CORPORATE SOURCE: Department of Molecular Biology, Biomedical Research Institute, Rockville, MD..
SOURCE: MOLECULAR AND BIOCHEMICAL PARASITOLOGY, (1991 Feb) 44 (2) 165-73.
Journal code: NOR. ISSN: 0166-6851.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199109

AB Plasmodium falciparum DNA is detected with an assay modeled according to the reverse target capture assay described by Morrissey et al. [19] for the detection of Listeria cells. A poly(A)-tailed oligonucleotide (pWZ34), derived from the partial sequence of a 4-kb repetitive unit of P. falciparum, functions as a capture probe and the labelled 21-bp repetitive units specific for P. falciparum serve as a **reporter** probe. Both probes are complementary to non-overlapping regions of the target DNA and in the presence of high concentration of chaotropic salts, hybridization efficiently takes place at relatively low temperatures (15 min. 37 degrees

C). The addition of poly(dT)-derivatized ferromagnetic beads allows the formation of A:T base pairing between the tailed beads and the tailed capture probe. Upon applying magnetic force, the target-capture-**reporter**-probe complex attached to the beads is removed from the reaction mixture, leaving the bulk of unreacted **reporter** molecules behind. Subsequent washings of the immobilized complex reduces the amount of non-specifically bound **reporter** probe. After elution of the complex from the beads a new cycle of capture, washing and release of the target-capture-**reporter**-probe complex is initiated by the additions of unused (dT)-tailed beads. After 3 cycles, the **signal**-to-**noise** ratio with 0.1 pg of P. falciparum DNA as a target was as high as 21-27, with a background of 8-10 cpm. The assay is unique in its speed, well suited for large sample numbers, and allows the manipulation of the background at will by simply increasing the number of capture rounds.

L13 ANSWER 14 OF 17 MEDLINE
ACCESSION NUMBER: 89028754 MEDLINE
DOCUMENT NUMBER: 89028754
TITLE: Detection of single fluorescent microtubules and methods for determining their dynamics in living cells.
AUTHOR: Sammak P J; Borisy G G
CORPORATE SOURCE: Laboratory of Molecular Biology, University of Wisconsin, Madison.
CONTRACT NUMBER: GM25062 (NIGMS)
SOURCE: CELL MOTILITY AND THE CYTOSKELETON, (1988) 10 (1-2) 237-45.
Journal code: CRD. ISSN: 0886-1544.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198902
AB The ability to tag biological molecules fluorescently and to detect their distribution in living cells has promoted the study of cytoplasmic organization in general and microtubule dynamics in particular. The techniques that we have selected and developed allowed the determination of spatial and temporal changes of the microtubule network in living fibroblasts at the level of individual microtubules. We have employed two general approaches for determining pattern changes: direct video microscopy and photobleaching and subsequent observation. Direct observation of fluorescent microtubules by high-definition video microscopy provided good spatial resolution at several time points, but was limited to the less congested and thinner periphery of the cell. This approach was made possible by a relatively bright, photostable **reporter**, xrhodamine-tubulin, and showed that microtubules underwent rounds of assembly and disassembly from their ends. Bleaching and subsequent observation of lysed cells improved the **signal** to **noise** ratio by extracting soluble chromophore and permitted observations in congested areas, but was limited to a single time interval. This approach demonstrated that microtubule domains were replaced one by one and that turnover was most rapid at the cell periphery. Antibodies specific for nonbleached chromophore can be used to enhance the **signal** to **noise** ratio further or to extend spatial resolution by the use of immunoelectron microscopy. Direct video microscopy and photobleaching are two approaches to the study of dynamics that have complementary strengths and wide application to the biology of living cells.

L13 ANSWER 15 OF 17 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1997:267009 BIOSIS
DOCUMENT NUMBER: PREV199799573612
TITLE: A highly sensitive cytotoxicity assay based on the release of **reporter** enzymes, from stably transfected cell lines.
AUTHOR(S): Schaefer, H. (1); Schaefer, A.; Kiderlen, A. F.; Masihi, K.
N.; Burger, R.

CORPORATE SOURCE: [redacted] Dep. Immunology, Robert Koch-[redacted]t., Nordufer 20, 13353 [redacted]lin Germany
SOURCE: Journal of Immunological Methods, (1997) Vol. 203, No. 1, pp. 89-98.
ISSN: 0022-1759.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The well-established methods of generating stably transfected cell lines, and the detection of nanomolar amounts of an enzyme in a fast and reproducible assay, were utilised to establish non-radiometric cytotoxicity assays. In these assay systems, the detection of released enzymes was used to quantitate the leakage of intracellular proteins after membrane disintegration. Target cell lines were transfected with a luciferase **reporter** gene under the control of a strong eucaryotic promoter. Release of the intracellular expressed enzyme into the culture supernatant occurred after membrane perforation and was measured as an indicator of cellular death. The quantitation of released enzyme was a reliable indicator of cell death initiated either by complement-mediated killing, or by cell-mediated cytotoxicity. This system was initially established with P815 mastocytoma cells as an example of a target cell line. Transfection with the firefly luciferase gene provided an intracellular enzyme absent in mammalian cells. In a parallel approach, P815 and BW5147 target cells were transfected with bacterial beta-galactosidase to provide a similar cytotoxicity system. This enzyme. however, has a considerably longer half life in tissue culture medium than luciferase. In a direct comparison between the standard 51Cr release and beta-galactosidase release, the enzyme release showed a much higher **signal**-to-**noise** ratio, i.e., low background and high induced release if spontaneous release and detergent induced maximal lysis were measured. Since a wide range of human and murine cell lines can be stably transfected and several **reporter** genes are available, the system should provide an alternative for conventional cytotoxicity assays.
The detection of released enzymes by colorimetric or luminometric methods makes this cytotoxicity assay independent of radionuclides. The sensitivity of luminometric enzyme detection systems should also permit the measurement of apoptotic processes and might make in vivo studies of cellular death using transgenic animals feasible.

L13 ANSWER 16 OF 17 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1989:268199 BIOSIS
DOCUMENT NUMBER: BA88:4281
TITLE: PEG-MEDIATED EXPRESSION OF GUS AND CAT GENES IN PROTOPLASTS FROM EMBRYOGENIC SUSPENSION CULTURES OF PICEA-GLAUCA.
AUTHOR(S): WILSON S M; THORPE T A; MOLONEY M M
CORPORATE SOURCE: PLANT PHYSIOL. RES. GROUP, DEP. BIOL. SCI., CALGARY, ALBERTA T2N 1N4, CAN.
SOURCE: PLANT CELL REP, (1989) 7 (8), 704-707.
CODEN: PCRPD8. ISSN: 0721-7714.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB .beta.-Glucuronidase (GUS) and chloramphenicol acetyl transferase (CAT) were used as **reporter** proteins in protoplasts from embryogenic suspension cultures of Picea glauca (Moench) Voss (white spruce). Plasmid DNA enclosing chimeric GUS and CAT constructs, using the cauliflower mosaic virus 35S promoter, was introduced into Picea gluca protoplasts using polyethylene glycol (PEG). Transient expression was detected 12 to 40 h after PEG-mediated DNA delivery. Dose-response curves using covalently closed circular plasmid DNA, in the absence of carrier DNA, have been obtianed for each of these **reporter** genes. Linearized plasmid DNA given lower levels of expression than covalently closed circular plasmid DNA when assayed 40 h after PEG-mediated DNA transfer.
The use of carrier DNA (herring sperm DNA), in combination with covalently

closed circular plasmid DNA, increased the level of expression of GUS by about 50%. CAT expression was echanced if PEG-mediated delivery was performed on ice rather than at room temperature. The highest level of expression for CAT, and the lowest **signal**-to-**noise** ratio, was found 24 h after PEG-mediated DNA transfer. Both GUS and CAT provided results that were quantifiable and can therefore be used as **reporter** genes in Picea glauca.

L13 ANSWER 17 OF 17 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
ACCESSION NUMBER: 2000:30168666 BIOTECHNO
TITLE: Evaluation of single nucleotide polymorphism typing with invader on PCR amplicons and its automation
AUTHOR: Mein C.A.; Barratt B.J.; Dunn M.G.; Siegmund T.; Smith A.N.; Esposito L.; Nutland S.; Stevens H.E.; Wilson A.J.; Phillips M.S.; Jarvis N.; Law S.; De Arruda M.; Todd J.
CORPORATE SOURCE: J. Todd, Wcome. Tst. Ctr. Std. Mol. Mec. Dis., University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2XY, United Kingdom. E-mail: john.todd@cimr.cam.ac.uk
SOURCE: Genome Research, (2000), 10/3 (330-343), 36 reference(s) CODEN: GEREFS ISSN: 1088-9051
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2000:30168666 BIOTECHNO
AB Large-scale pharmacogenetics and complex disease association studies will require typing of thousands of single-nucleotide polymorphisms (SNPs) in thousands of individuals. Such projects would benefit from a genotyping system with accuracy > 99% and a failure rate < 5% on a simple, reliable, and flexible platform. However, such a system is not yet available for routine laboratory use. We have evaluated a modification of the previously reported Invader SNP-typing chemistry for use in a genotyping laboratory and tested its automation. The Invader technology uses a Flap Endonuclease for allele discrimination and a universal fluorescence resonance energy transfer (FRET) **reporter** system. Three hundred and eighty-four individuals were genotyped across a panel of 36 SNPs and one insertion/deletion polymorphism with Invader assays using PCR product as template, a total of 14,208 genotypes. An average failure rate of 2.3% was recorded, mostly associated with PCR failure, and the typing was 99.2% accurate when compared with genotypes generated with established techniques. An average **signal-to-noise** ratio (9:1) was obtained. The high degree of discrimination for single base changes, coupled with homogeneous format, has allowed us to deploy liquid handling robots in a 384-well microtitre place format and an automated end-point capture of fluorescent signal. Simple semiautomated data interpretation allows the generation of .sim.25,000 genotypes per person per week, which is 10-fold greater than gel-based SNP typing and microsatellite typing in our laboratory. Savings on labor costs are considerable. We conclude that Invader chemistry using PCR products as template represents a useful technology for typing large numbers of SNPs rapidly and efficiently.

=> d his

(FILE 'HOME' ENTERED AT 11:25:03 ON 14 MAY 2000)

FILE 'BIOBUSINESS, LIFESCI, CAPLUS' ENTERED AT 11:27:47 ON 14 MAY 2000

FILE 'BIOBUSINESS, BIOTECHDS' ENTERED AT 11:32:12 ON 14 MAY 2000

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L1          224 S HI[redacted]THROUGHPUT
L2         4290 S RE[redacted]RTER
L3           14 S L1 AND L2
L4           12 DUP REM L3 (2 DUPLICATES REMOVED)

     FILE 'MEDLINE, BIOSIS, BIOTECHNO' ENTERED AT 11:44:03 ON 14 MAY 2000
L5         3206 S HIGH THROUGHPUT
L6         9713 S (SIGNAL TO NOISE)
L7           19 S L5 AND L6
L8           12 DUP REM L7 (7 DUPLICATES REMOVED)
L9        50715 S REPORTER
L10       50715 S L2 OR L9
L11          39 S L9 AND L6
L12          18 DUP REM L11 (21 DUPLICATES REMOVED)
L13          17 S L12 NOT L8

=> s assay or screen?

L14     936269 ASSAY OR SCREEN?

=> s l14 and l6

L15       1034 L14 AND L6

=> s promoter

L16     188856 PROMOTER

=> s l15 and l16

L17         11 L15 AND L16

=> dup rem l17

PROCESSING COMPLETED FOR L17
L18          6 DUP REM L17 (5 DUPLICATES REMOVED)

=> d ibib abs 1-6


L18  ANSWER 1 OF 6  MEDLINE                              DUPLICATE 1
ACCESSION NUMBER:   2000011244     MEDLINE
DOCUMENT NUMBER:    20011244
TITLE:              Stress responses as a tool To detect and characterize the
                    mode of action of antibacterial agents.
AUTHOR:             Bianchi A A; Baneyx F
CORPORATE SOURCE:   Department of Bioengineering, University of Washington,
                    Seattle, Washington 98195, USA.
SOURCE:             APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1999 Nov) 65 (11)
                    5023-7.
                    Journal code: 6K6. ISSN: 0099-2240.
PUB. COUNTRY:       United States
                    Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200002
ENTRY WEEK:         20000204
AB   Single-copy gene fusions between the lacZ reporter gene and Escherichia
     coli strains containing promoters induced by cold shock (cspA),
     cytoplasmic stress (ibp), or protein misfolding in the cell envelope
     (P3rpoH) were constructed and tested to determine their ability to detect
     antibacterial agents while simultaneously providing information on their
     cellular targets. Antibiotics that affect prokaryotic ribosomes
     selectively induced the cspA::lacZ or ibp::lacZ gene fusion, depending on
     their mode of action. The membrane-damaging peptide polymyxin B induced
     both the P3rpoH::lacZ and ibp::lacZ fusions, while the beta-lactam
     antibacterial agent carbenicillin activated only the P3rpoH
     promoter. Nalidixic acid, a compound that causes DNA damage,
     downregulated beta-galactosidase synthesis from P3rpoH but had little
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effect on expr[redacted]on of the reporter enzyme fro[redacted]ither the cspA or ibp **promoter**. All m[redacted]el antibiotics could be identif[redacted]d over a wide range of sublethal concentrations with **signal**-to-**noise** ratios between 2 and 11. A blue halo **assay** was developed to rapidly characterize the modes of action of antibacterial agents by visual inspection, and this **assay** was used to detect chloramphenicol secreted into the growth medium of Streptomyces venezuelae cultures. This simple system holds promise for **screening** natural or combinatorial libraries of antimicrobial compounds.

L18 ANSWER 2 OF 6 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1999:427763 BIOSIS
DOCUMENT NUMBER: PREV199900427763
TITLE: Two-stage transformation **assay** for cigarette smoke condensates using murine C3H-10T1/2 fibroblasts.
AUTHOR(S): Schlage, W. K. (1); Buelles, H.; Friedrichs, D.; Kurkowsky, B.
CORPORATE SOURCE: (1) INBIFO Institut fuer biologische Forschung, Fuggerstr. 3, 51149, Koeln Germany
SOURCE: Toxicology In Vitro, (Aug. Oct., 1999) Vol. 13, No. 4-5, pp. 823-828.
ISSN: 0887-2333.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We investigated whether the two-stage transformation **assay** can be applied in routine testing for **promoter**-like activity of cigarette smoke condensate (CSC) as an in vitro equivalent of an in vivo tumorigenicity **assay** (mouse skin painting). We adopted a published **assay** procedure (Frazelle et al., 1983a), using 3-methylcholanthrene (MCA, 0.37 mumol/litre, 24 hr treatment) as the initiator. Rigorously standardized experimental conditions, such as multiparameter-**screened** serum, one fixed subculture level, and a rigid preculturing schedule, were employed. Transformation was expressed as the fraction of dishes containing type II and type III foci. Compared to the positive control, 12-O-tetradecanoylphorbol-13-acetate (TPA), transformation responses to CSC (three CSC batches that had different in vivo activity) were lower. Variations in dose-response relationships did not allow distinction between two of the three CSC batches, even with data pooled from seven **assay** repetitions over 2 years. In a second approach, to enhance the **assay** resolution, that is, the **signal**-to-**noise** ratio, **promoter** treatment twice per week was ineffective: the response and the background were both increased. Lowering the initiator concentration (0.08 mumol/litre) enhanced the **signal**-to-**noise** ratio for TPA, but not for CSC. Even after standardization and enhancement of sensitivity, the two-stage transformation **assay** is useful primarily for qualitative assessment of **promoter**-like activity of weak promoters, such as CSC, rather than for quantitative comparisons.

L18 ANSWER 3 OF 6 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1998447473 MEDLINE
DOCUMENT NUMBER: 98447473
TITLE: A highly sensitive and specific **assay** using a novel human growth hormone cDNA reporter gene regulated by the human interleukin-4 inducible germline epsilon transcript **promoter**.
AUTHOR: Jenh C H; Cox M A; Lundell D; Narula S K; Zavodny P J
CORPORATE SOURCE: Department of Immunology, Schering-Plough Research Institute, Kenilworth, NJ 07033, USA.. chung-her.jenh@spcorp.com
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1998 Aug 1) 217 (1-2) 87-95.
Journal code: IFE. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199901
ENTRY WEEK: 19990104
AB We have successfully developed a highly sensitive and specific **assay** system for human interleukin-4 (IL-4) regulated gene expression. It is based on a human Jijoye cell line with the germline epsilon transcript **promoter** joined to the human growth hormone (hGH) cDNA. The germline epsilon transcript **promoter** is responsive to IL-4 and involved in immunoglobulin heavy chain class switching. We cloned hGH complementary DNA (cDNA) as the reporter gene instead of using conventional hGH genomic DNA which failed to generate any IL-4 inducible clone in human Jijoye cells. The two IL-4 inducible cell lines with the hGH cDNA reporter show high **signal/noise** ratio for IL-4-mediated induction (60-90 fold). The response to IL-4 is dose-dependent with ED50 of 10 pM. As expected, there is no response to other human cytokines and growth factors, as well as mouse IL-4. The mutant hIL-4 antagonist hIL-4.Y124D inhibits the induction mediated by native hIL-4. These IL-4 inducible cell lines provide a sensitive, specific **assay** system to study IL-4-regulated gene expression, and in particular the regulation of the germline epsilon **promoter** .

L18 ANSWER 4 OF 6 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 97346293 MEDLINE
DOCUMENT NUMBER: 97346293
TITLE: A highly sensitive cytotoxicity **assay** based on the release of reporter enzymes, from stably transfected cell lines.
AUTHOR: Schafer H; Schafer A; Kiderlen A F; Masihi K N; Burger R
CORPORATE SOURCE: Department of Immunology, Robert Koch-Institute, Berlin, Germany.
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1997 May 12) 204 (1) 89-98.
Journal code: IFE. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199709
ENTRY WEEK: 19970904
AB The well-established methods of generating stably transfected cell lines, and the detection of nanomolar amounts of an enzyme in a fast and reproducible **assay**, were utilised to establish non-radiometric cytotoxicity assays. In these **assay** systems, the detection of released enzymes was used to quantitate the leakage of intracellular proteins after membrane disintegration. Target cell lines were transfected with a luciferase reporter gene under the control of a strong eucaryotic **promoter**. Release of the intracellular expressed enzyme into the culture supernatant occurred after membrane perforation and was measured as an indicator of cellular death. The quantitation of released enzyme was a reliable indicator of cell death initiated either by complement-mediated killing, or by cell-mediated cytotoxicity. This system was initially established with P815 mastocytoma cells as an example of a target cell line. Transfection with the firefly luciferase gene provided an intracellular enzyme absent in mammalian cells. In a parallel approach, P815 and BW5147 target cells were transfected with bacterial beta-galactosidase to provide a similar cytotoxicity system. This enzyme, however, has a considerably longer half life in tissue culture medium than luciferase. In a direct comparison between the standard 51Cr release and beta-galactosidase release, the enzyme release showed a much higher **signal**-to-**noise** ratio, i.e., low background and high induced release if spontaneous release and detergent induced maximal lysis

were measured. Since a wide range of human and murine cell lines can be stably transfected and several reporter genes are available, the system should provide an alternative for conventional cytotoxicity assays. The detection of released enzymes by colorimetric or luminometric methods makes this cytotoxicity **assay** independent of radionuclides. The sensitivity of luminometric enzyme detection systems should also permit the measurement of apoptotic processes and might make in vivo studies of cellular death using transgenic animals feasible.

L18 ANSWER 5 OF 6 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1997:267009 BIOSIS
DOCUMENT NUMBER: PREV199799573612
TITLE: A highly sensitive cytotoxicity **assay** based on the release of reporter enzymes, from stably transfected cell lines.
AUTHOR(S): Schaefer, H. (1); Schaefer, A.; Kiderlen, A. F.; Masihi, K.
N.; Burger, R.
CORPORATE SOURCE: (1) Dep. Immunology, Robert Koch-Inst., Nordufer 20, 13353 Berlin Germany
SOURCE: Journal of Immunological Methods, (1997) Vol. 203, No. 1, pp. 89-98.
ISSN: 0022-1759.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The well-established methods of generating stably transfected cell lines, and the detection of nanomolar amounts of an enzyme in a fast and reproducible **assay**, were utilised to establish non-radiometric cytotoxicity assays. In these **assay** systems, the detection of released enzymes was used to quantitate the leakage of intracellular proteins after membrane disintegration. Target cell lines were transfected with a luciferase reporter gene under the control of a strong eucaryotic **promoter**. Release of the intracellular expressed enzyme into the culture supernatant occurred after membrane perforation and was measured as an indicator of cellular death. The quantitation of released enzyme was a reliable indicator of cell death initiated either by complement-mediated killing, or by cell-mediated cytotoxicity. This system was initially established with P815 mastocytoma cells as an example of a target cell line. Transfection with the firefly luciferase gene provided an intracellular enzyme absent in mammalian cells. In a parallel approach, P815 and BW5147 target cells were transfected with bacterial beta-galactosidase to provide a similar cytotoxicity system. This enzyme. however, has a considerably longer half life in tissue culture medium than luciferase. In a direct comparison between the standard 51Cr release and beta-galactosidase release, the enzyme release showed a much higher **signal**-to-**noise** ratio, i.e., low background and high induced release if spontaneous release and detergent induced maximal lysis were measured. Since a wide range of human and murine cell lines can be stably transfected and several reporter genes are available, the system should provide an alternative for conventional cytotoxicity assays. The detection of released enzymes by colorimetric or luminometric methods makes this cytotoxicity **assay** independent of radionuclides. The sensitivity of luminometric enzyme detection systems should also permit the measurement of apoptotic processes and might make in vivo studies of cellular death using transgenic animals feasible.

L18 ANSWER 6 OF 6 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 94327718 MEDLINE
DOCUMENT NUMBER: 94327718
TITLE: Detection of herpes simplex virus by measurement of luciferase activity in an infected-cell lysate.
AUTHOR: Olivo P D
CORPORATE SOURCE: Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110..

SOURCE: [redacted]URNAL OF VIROLOGICAL METHODS, [redacted]94 Apr) 47 (1-2) 117-28.
Journal code: HQR. ISSN: 0166-0934.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199411

AB A stably transformed cell line (BHKICP6LucA6) has been isolated which expresses high levels of luciferase activity following infection with herpes simplex virus (HSV). The genome of this cell line contains an HSV-1 **promoter**-luciferase chimeric gene. Infected BHKICP6LucA6 cells exhibit a level of luciferase activity 5 x 10(5) higher than mock-infected cells. This **signal**-to-**noise** ratio is of a sufficient magnitude that measurement of the luciferase activity of an infected-cell lysate can detect a single infected cell when a practical number of cells is used in the **assay**. This approach to the detection of infectious virus could be useful in a number of circumstances and may be adaptable to an automated **assay** which could become a useful means for diagnostic laboratories to detect viruses in clinical specimens.

STIC-ILL

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Subject: 09/378046

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1039

ACCESSION NUMBER: 97155512 MEDLINE
DOCUMENT NUMBER: 97155512
TITLE: Genetically modified Escherichia coli for colorimetric detection of inorganic and organic Hg compounds.
AUTHOR: Klein J; Altenbuchner J; Mattes R
CORPORATE SOURCE: Institute of Industrial Genetics, University of Stuttgart, Germany.
SOURCE: EXS, (1997) 80 133-51.
Journal code: BFZ.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY WEEK: 199704

ND 5/15

TITLE: Comparison of mutant forms of the green fluorescent protein as expression markers in Chinese hamster ovary (CHO) and Saccharomyces cerevisiae cells.

ZC6UU5157058

AUTHOR: Natarajan A; Subramanian S; Srienc F
CORPORATE SOURCE: Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, USA.
SOURCE: JOURNAL OF BIOTECHNOLOGY, (1998 Jun 11) 62 (1) 29-45.
Journal code: AL6. ISSN: 0168-16

TITLE: A highly sensitive and specific assay using a novel human growth hormone cDNA ***reporter*** gene regulated by the human interleukin-4 inducible germline epsilon transcript promoter.
AUTHOR: Jenh C H; Cox M A; Lundell D; Narula S K; Zavodny P J
CORPORATE SOURCE: Department of Immunology, Schering-Plough Research Institute, Kenilworth, NJ 07033, USA.. chung-her.jenh@spcorp.com
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1998 Aug 1) 217 (1-2) 87-95.
Journal code: IFE. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
Journal;

TITLE: Development of a green fluorescent protein ***reporter*** for a yeast genotoxicity biosensor.
AUTHOR: Billinton N; Barker M G; Michel C E; Knight A W; Heyer W D; Goddard N J; Fielden P R; Walmsley R M
CORPORATE SOURCE: Department of Biomolecular Sciences, UMIST, Manchester, UK.
SOURCE: BIOSENSORS AND BIOELECTRONICS, (1998 Oct 1) 13 (7-8) 831-8. ISSN: 0956-5663

Frontiers in Biosensorics I
Fundamental Aspects
ed. by F.W. Scheller, F. Schubert and J. Fedrowitz

Genetically modified *Escherichia coli* for colorimetic detection of inorganic and organic Hg compounds

J. Klein, J. Altenbuchner and R. Mattes

Institute of Industrial Genetics, University of Stuttgart, D-70569 Stuttgart, Germany.

Summary: A sensitive colorimetric bacterial system was developed for the detection of Hg(II) and organomercury compounds. The bioactive species, a recombinant *Escherichia coli*, produces proportionally elevated levels of the enzyme β-galactosidase with increasing amounts of Hg. This is due to a reporter plasmid which carries a Hg(II)-inducible promoter (*mer* promoter) from the Hg resistance transposon Tn*501* regulating the transcription of a promoterless *lacZ* gene. Additionally, a pMB1 origin of replication without the natural RNA polymerase start site is fused downstream of the *mer* promoter leading to a Hg(II)-inducible plasmid replication, which results in an improved signal-to-noise ratio. To enhance the sensitivity of this cellular biosensor, the transport proteins for Hg(II) uptake are constitutively produced by a helper plasmid. To enable the detection of organically bound Hg, the *Streptomyces lividans* organomercurial lyase, an enzyme which catalyses the cleavage of C-Hg-bonds of organomercurial compounds, is also provided by the helper pasmid. Hg(II) and phenylmercuric acetate (PMA) concentrations as low as 5×10^{-10} M (0.1 ppb) may be detected within a few minutes.

Introduction

The response of biological systems to compounds within the environment has been a conceptual basis for the development of bioassays. An example is the inhibition of growth due to toxic substances which allows an accurate biospecific determination of concentrations of e.g. antibiotics (minimal inhibitory concentration). But these methods are tedious and time consuming and often not specific, at least when mixtures of unknown composition have to be tested. In addition some biological systems have acquired resistance mechanisms to overcome life-threatening effects of toxic compounds. Four principal biological strategies are observed which are summarised in Figure 1. These include passive strategies, for example a general change in permeability which reduces uptake, or a mutation in the gene encoding the target component may render the cell resistant. In contrast, active strategies involve the acquistion of additional genes leading to the production of new enzymes, which metabolise and thus inactivate the toxic component or facilitate its export.

Generally the latter active mechanisms of resistance are highly specific and include the production of distinct proteins, mainly enzymes, which interact with the substance in question. Biochemical methods measuring

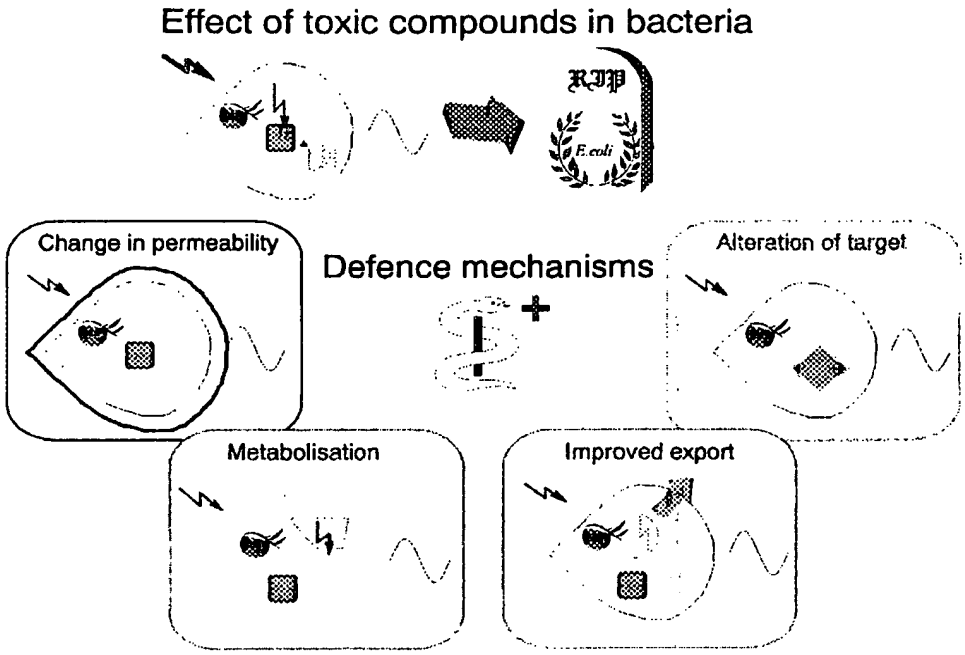


Figure 1. Schematic view of possible defence mechanisms for microbial resistance to toxic compounds.

the activity of such specific enzymes allowed the development of faster and more precise alternative tests rather than merely monitoring growth inhibition. In consequence it is also conceivable to quantify specific intracellular reactions which lead to growth retardation alternatively. These modern possibilities for qualitative or quantitative assays with microbial cells allow the development of biospecific microbial sensors.

When studying active resistance mechanisms of microbial cells it became apparent that the genetic determinants involved are usually regulated. The production of encoded specific proteins is induced in response to the toxic substance on a level which is usually concentration dependent. The application of reporter genes using gene fusions allowed to identify the regulatory components of gene expression, a method now widely used in such research. We attempted to redirect the aim of reporter gene analysis for the understanding of regulatory mechanisms into the modular development of specific biosensoric cells. For this purpose we evaluated the genetic modules necessary for accurate and concentration-dependent production of reporter enzymes with respect to the inducing agent.

As a model system the genetic components for resistance against mercury compounds were used. These are most important heavy metal environmental pollutants in form of inorganic and organic Hg compounds, released by a wide spectrum of industrial and agricultural processes or by

the weathering of Hg-bearing rocks (Robinson and Tuovinen, 1984). Human exposure to even small amounts (ng to μg) of Hg compounds, especially the more toxic organomercurials, leads to nervous and physical disorders (Aschner and Aschner, 1990; De Flora et al., 1994). The need for analysis and monitoring environmental and food contamination by Hg compounds led to the development of sensitive and reliable physicochemical detection procedures like atomic absorption spectrophotometry (Omang, 1971), or more sensitive, cold vapour atomic fluorescence detection (Bloom and Fitzgerald, 1988). Although these detection methods are highly sensitive, they have some disadvantages: sample preparation, extraction and interpretation is laborious and involves expensive equipment and highly skilled staff. This makes it impossible to perform measurements without delay (*on line*) at the site of interest (*on site*). Simple biological systems based on proteins with high affinity to Hg compounds may contribute to fill this gap between time-consuming Hg work-up protocols and the desirable *on site* and *on line* measurement of Hg. One of these biological approaches is the use of specific antibodies in an ELISA-test system to detect Hg(II) (Wylie et al., 1991). A further way may be the development of specific cellular Hg biosensors (Klein et al., 1989; 1991; Selifonova et al., 1993; Tescione and Belfort, 1993) based on bacterial Hg resistance mechanisms.

Specific Hg detoxification systems are present in many Gram-negative and Gram-positive bacteria isolated from polluted areas (Silver et al., 1989; Silver and Walderhaug, 1992). Although they belong to very different taxonomic groups the microorganisms display similar resistance mechanisms encoded by different but homologous genes which always are organised in regulated operon entities (Fig. 2). The resistance mechanism is based on the effective import of Hg(II) into the bacterial cell by transport proteins (MerT and MerP) and immediate enzymatic detoxification by reduction of the ions via a mercuric reductase (MerA) to the less toxic and volatile Hg (Summers, 1986). Some organisms are even able to deal with a broad range of organomercurial compounds. The organomercurial lyase (MerB) cleaves the C-Hg bond of organomercurials and releases Hg(II), which is subsequently reduced by MerA (Fig. 3). The various *mer* genes are arranged in an operon, whereby transcription is controlled by a regulatory protein (MerR) which specifically recognises Hg(II). It may act as a positive activator of transcription in the presence of Hg(II) and as a repressor in their absence, as exemplified for Tn*501* (Lund and Brown, 1989; O'Halloran et al., 1989) (Fig. 4). In contrast, for the operon of *Streptomyces lividans* MerR was shown to act solely as repressor, whose action is relieved by the inducer compound Hg(II) (Brünker et al., 1996).

Concerning their efforts to elucidate the regulation mechanism of the narrow spectrum resistance determinants of the transposon Tn*501* or the similar Tn*21* (Silver et al., 1989), Lund and Brown (1989) or Ross et al. (1989), Park et al. (1992) and Condee and Summers (1992) have used

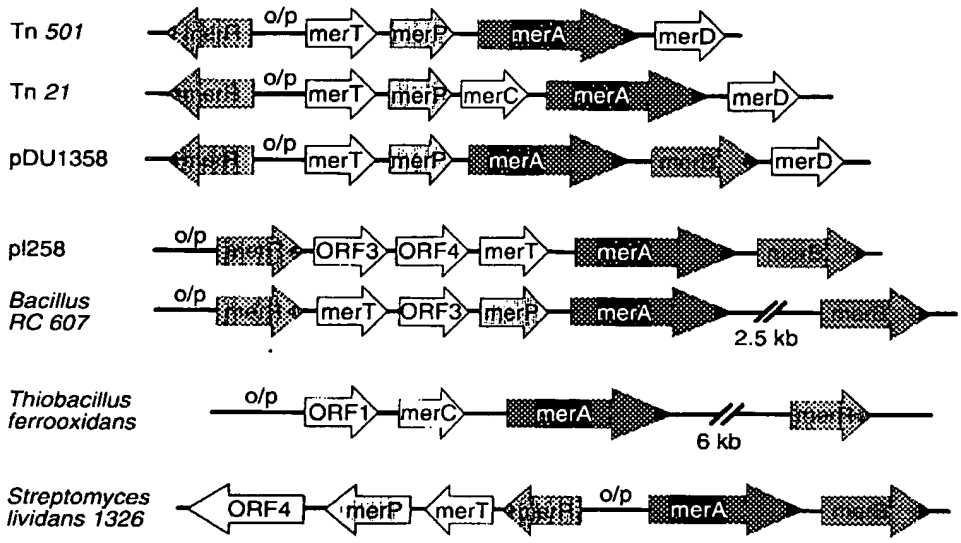


Figure 2. Organization of genes in bacterial mercury resistance operons.

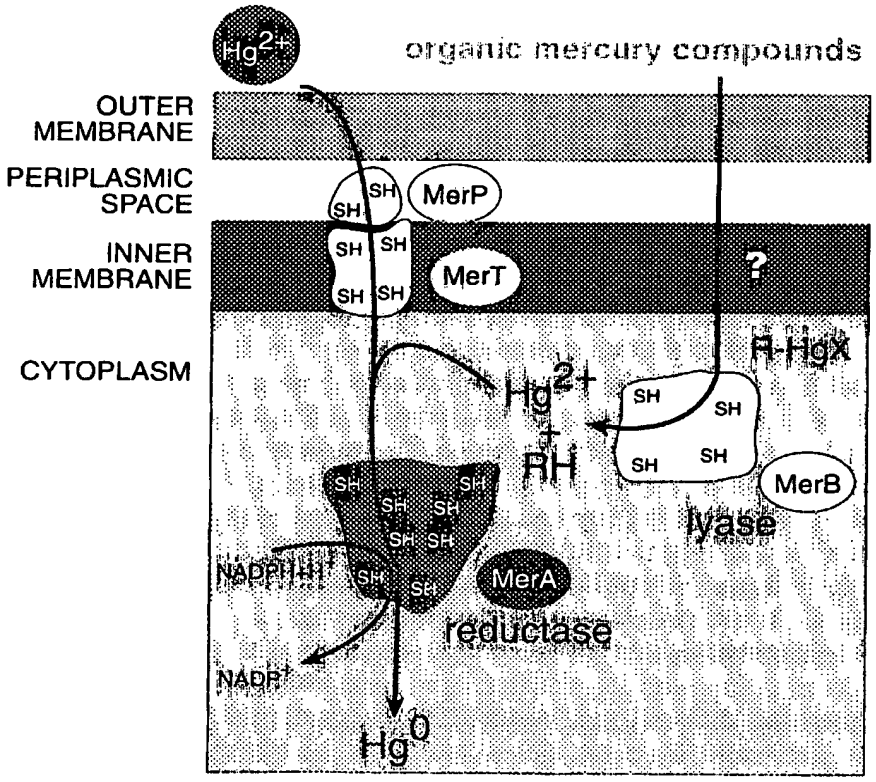


Figure 3. Detoxification of mercurial compounds in gram-negative bacteria.

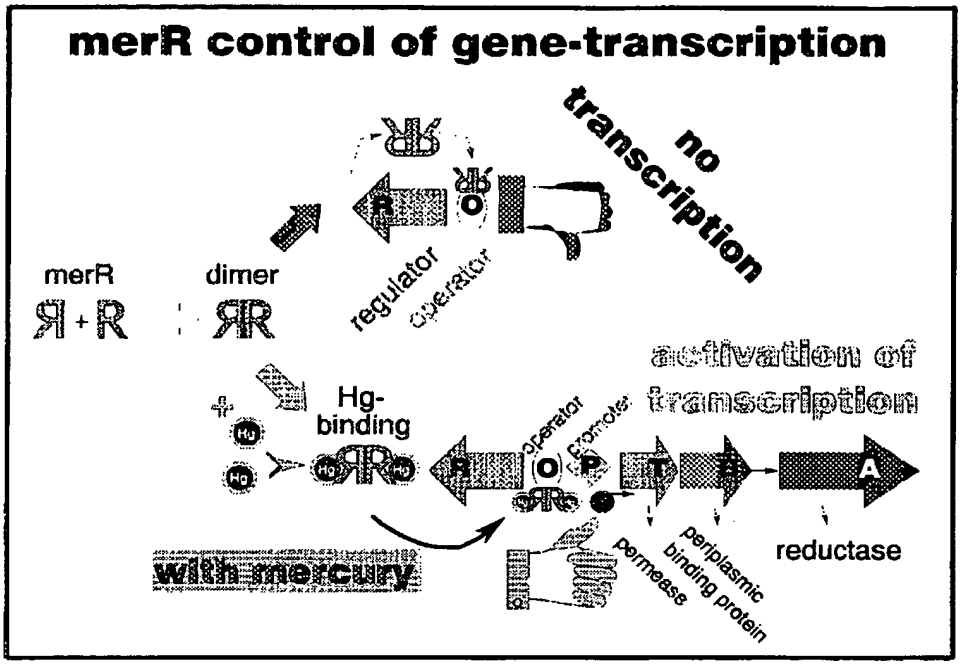


Figure 4. Transcriptional control of *mer* operon expression by the regulator protein MerR. Repression by MerR dimer but activation by Hg(II)-[MerR]$_2$-complex.

transcriptional fusions with reporter genes such as *lacZ* or *lux*. The transcriptional fusion of a reporter gene to a *mer* operon, for example to the well-known Tn*501* operon, creates a bacterial strain which is able to monitor the presence of Hg(II) by the production of quantitatively detectable reporter enzyme activities. In that way luminometric biosensors specific for Hg(II) were described by Tescione and Belfort (1993) or Selifonova et al. (1993). The investigations presented here aimed at the systematic improvement of a colorimetric Hg-specific bacterial biosensor.

Materials and methods

Bacterial strains, media and growth conditions

All cloning and induction experiments were carried out in *Escherichia coli*, strain JM109 (*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ (*lac-pro-AB*) F'[*traD36 proAB*$^+$ *lacI*^{q} *lacZ*ΔM15], Yanisch-Perron et al., 1985) using 2 YT medium (16 g/l tryptone, 10 g/l yeast extract, 5 g/l NaCl). The following antibiotics were used for selection and/or maintenance of plasmids in JM109: 100 µg/ml ampicillin, 25 µg/ml chloramphenicol, 50 µg/ml kanamycin or 20 µg/ml tetracycline.

DNA manipulations and cell transformation

All DNA manipulations were carried out as described elsewhere (Sambrook et al., 1989). All enzymes were purchased from Boehringer Mannheim/ Germany, except *Taq* DNA polymerase from Pharmacia LKB GmbH, Freiburg/Germany and used according to the manufacturers' instructions. *E. coli* transformations were performed using the TSS method, originally described by Chung et al. (1989).

Plasmids

All used plasmids are summarised in Table 1. pJOE115 is a pBR322-derivative carrying the Hg resistance transposon (Altenbuchner et al., 1981). A 2340 bp *Eco*RI fragment from pJOE115 containing *merR*, the regulatory region *op*, *merT* and *merP* (*merRopTP* cassette) was integrated into the *Eco*RI restriction site of plasmid pKO4 (McKenney et al., 1981) to obtain pJKS1. A promoterless 3811 bp *lacZ* gene of pRU676::Tn*1731* (Ubben and Schmitt, 1987) was transferred as a *Bam*HI fragment into the *Bam*HI-digested plasmid pJKS1 to create pJKS3. pJKS19 was derived from pUC19 (Vieira and Messing, 1982) by inserting the above-mentioned *merRopTP* cassette and *Bam*HI *lacZ* fragments into the appropriate restriction sites. pJKS68 is a derivative of pJKS3. The origin of replication of pBR322 lacking the appropriate RNA polymerase start site was first inserted into *Nru*I-digested pIC19 as a 434 bp *Hae*III fragment, and from there transferred as a 467 bp *Eco*RI-*Hin*dIII fragment into *Eco*RI-*Hin*dIII-cut pKO4. The *Eco*RI *merRopTP* cassette fragment (2340 bp) and the *Bam*HI *lacZ* fragment (3811 bp) were successively inserted into this plasmid resulting in the reporter plasmid pJKS68. A 686 bp *Eco*RI-*Ava*I fragment of pJOE115, which carried *merRopT'*, was isolated and inserted into a helper plasmid which provide *merRopT''*as *Eco*RI fragment (Klein, 1992). This 702 bp *Eco*RI fragment containing only a functional *merR* gene and the regulatory region was inserted into *Eco*RI-digested pKO4, followed by cloning of the *Bam*HI *lacZ* fragment (3811 bp) into this plasmid to give pJKS39. The genes *merT* and *merP* were obtained as a 993 bp *Bgl*I fragment of pJOE115. Sticky ends were filled in with Klenow polymerase and the fragment was blunt-end ligated into *Eco*RV-cut pACYC184 (Chang and Cohen, 1978) to yield pJKS13. The gene *merB* was isolated as a 655 bp *Bam*HI fragment from pJOE851-461 (Sedlmeier and Altenbuchner, 1992) via PCR and ligated to *Bam*HI-digested pJKS13 to obtain pJOE2004.

Hg-specific β-galactosidase induction in liquid cultures

E. coli strains bearing the constructed plasmids were grown overnight in 2YT medium at 37°C with the appropriate antibiotics, diluted 1 : 100

Table 1. Plasmids used in this study

Plasmid	Resistance[a]	Relevant genotype and features[b]	Origin of replication	Source (reference)
pACY184	Cm^{r}Tc^r	–	p15A	Chang and Cohen, 1978
pACYC184::Tn*21*	Cm^{r}Tc^r	Tn*21* in pACYC184	p15A	De la Cruz and Grinstedt, 1982
pBR322	Ap^{r}Tc^r	–	pMB1	Bolivar et al., 1977
pIC19H	Ap^r	*rop*$^-$	pMB1	Marsh et al., 1984
pJOE115	Ap^r	Tn*501* in pJOE100	pMB1	Altenbucher, unpublished
pJOE851-461	Ap^r	*merRAB (S. lividans)*	pMB1	Sedlmeier and Altenbuchner, 1992
pKO4	Ap^r	*galK*	pMB1	McKenney et al., 1981
pRU6776::Tn*1731*	Km^r	*aphA lacZ* in Tn*1731*	pMB1	Ubben and Schmitt, 1987
pUC18	Ap^r	*rop*	pMB1	Vieira and Messing, 1982
pJKS3	Ap^r	*merRopTP lacZ*	pMB1	this study
pJKS13	Cm^r	*ptetA meropTP*	p15A	this study
pJKS19	Ap^r	*merRopTP lacZ*	pMB1	this study
pJKS68	Ap^r	*merRopTP lacZ ori*	pMB1	this study
pJKS81	Cm^r	*ptetA meropT-lacZ*	p15A	this study
pJOE2004	Cm^r	*ptetA meropTP B*	p15A	this study

[a] Ap^r: ampicillin resistance, Cm^r: chloramphenicol resistance, Km^r: kanamycin resistance, Tc^r: tetracycline resistance.
[b] *op*: operator/promoter region of Tn*501*, *TP*: *merTP* of Tn*501*, *T-lacZ*: translational fusion of MerT and LacZ, *ori*: origin of replication of pBR322, *B*: *merB* of *Streptomyces lividans*, inactivated or deleted genes are not mentioned.

in fresh medium and cultivated to the late exponential growth phase (A_{600} = 0.7). 2 ml of the growing cultures were transferred to 10-ml test tubes and different concentrations of Hg(II), phenylmercury acetate or other heavy metal salts were added for induction. The cultures were further incubated at 37 °C for fifteen minutes or as otherwise indicated. β-galactosidase (β-gal) assays were immediately carried out as described by Miller (1972). The activities are the mean values of three independent measurements with standard deviations of less than 10%. The detection threshold was defined as the Hg concentration which led to a twofold increase in activity compared to uninduced β-gal activity.

Results

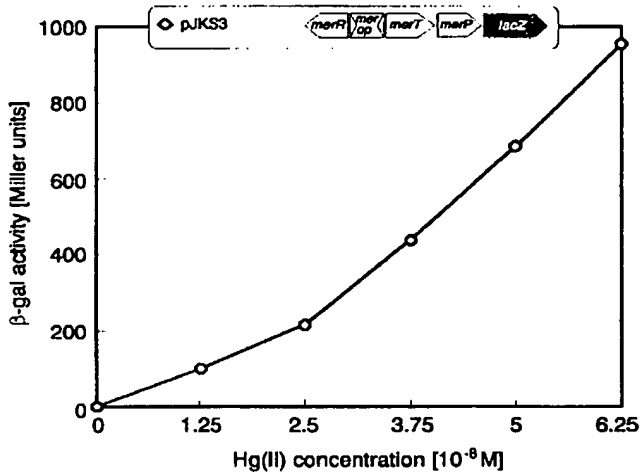
Transcriptional fusion of lacZ as reporter gene to the mer genes of Tn501

The concentration of Hg(II) can be determined via a reporter enzyme activity whose production is dependent on a transcriptional fusion of the reporter gene to a Hg resistance operon (Klein et al., 1989; Tescione and Belfort, 1993; Selifonova, 1993). For convenience and to provide a broad application of the resulting Hg specific biosensor, there should be a rapid and simple colorimetric assay to measure the activity of the reporter enzyme. Furthermore, low concentrations of Hg(II), Hg(I) or organomercurials should not inhibit the enzyme. Both requirements were met by the β-galactosidase (β-gal) of *E. coli*. A series of chromogenic and even highly sensitive chemiluminescent substrates are available and, using purified β-gal, it was confirmed that enzyme activity is not influenced by Hg(II) concentrations as high as 5×10^{-6} M (1000 ppb) (data not shown).

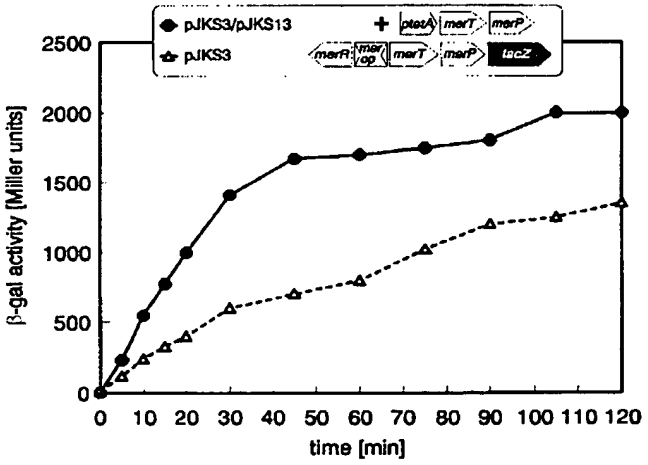
For transcriptional fusion of the *lacZ* gene to the *mer* operon of Tn*501*, observations of Nakahara et al. (1979) and Lund and Brown (1987) were taken into account who showed that the presence of intact Hg transport and regulatory proteins without a functional reductase led to Hg(II) supersensitive cells. Without reduction to the volatile Hg(0), the *mer* operon inducing Hg(II) were efficiently imported into the cells and accumulated. Consequently, a promoterless reporter gene fused within *merA* downstream of *merR*, the regulatory region and *merT* and *merP* of Tn*501* was expected to be induced even at very low concentrations of Hg(II). A *merA* deleted 2340 pb *Eco*RI fragment of Tn*501* which contained the transport protein genes *merT* and *merP* and the regulatory elements was inserted upstream of a promoterless *lacZ* gene on the high copy plasmid pKO4 to create pJKS3 (Tab. 1). Translation stop codons in all three reading frames at the 5′ end of *lacZ* prevented translational fusions. To analyse induction of *E. coli* JM109 (pJKS3) by Hg(II), the strain was incubated for one hour with fairly low concentrations of $HgCl_2$ up to 6.25×10^{-8} M (12.5 ppb) and the β-gal activities were determined. As illustrated in Figure 5 A, a linear correlation of Hg(II) concentration and enzyme activity could be shown in the investigated concentration range. The detection threshold was about 10^{-8} M (2 ppb).

Figure 5. Genetic factors influencing the biosensor sensitivity. (A) Hg(II)-dependent β-gal synthesis of *E. coli* JM109 (pJKS3). (B) Influence of the transport protein level on the β-gal production after induction with 10^{-7} M (20 ppb) Hg(II). (C) Influence of the copy number of the reporter plasmid. All strains were cultivated to the exponential growth phase in 2YT and induced with the indicated Hg(II) concentrations. After different induction times [(A) 60 min, (B) 5 120 min. (C) 15 min], the β-gal activities were measured. The insets show the molecular organization of the test plasmids.

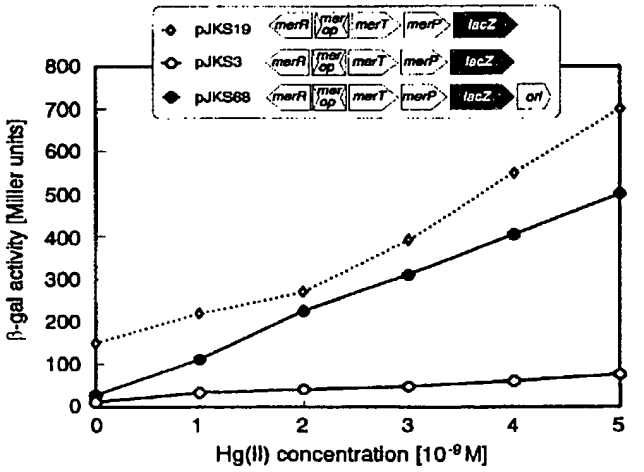
(A)


pJKS3
merR
mer op
merT
merP
lacZ
β-gal activity [Miller units]
1000
800
600
400
200
0
0
1.25
2.5
3.75
5
6.25
Hg(II) concentration [10⁻⁹ M]

(B)


pJKS3/pJKS13
+
ptetA
merT
merP
pJKS3
merR
mer op
merT
merP
lacZ
β-gal activity [Miller units]
2500
2000
1500
1000
500
0
0 10 20 30 40 50 60 70 80 90 100 110 120
time [min]

(C)


pJKS19
pJKS3
pJKS68
merR
mer op
merT
merP
lacZ
ori
β-gal activity [Miller units]
800
700
600
500
400
300
200
100
0
0
1
2
3
4
5
Hg(II) concentration [10⁻⁹ M]

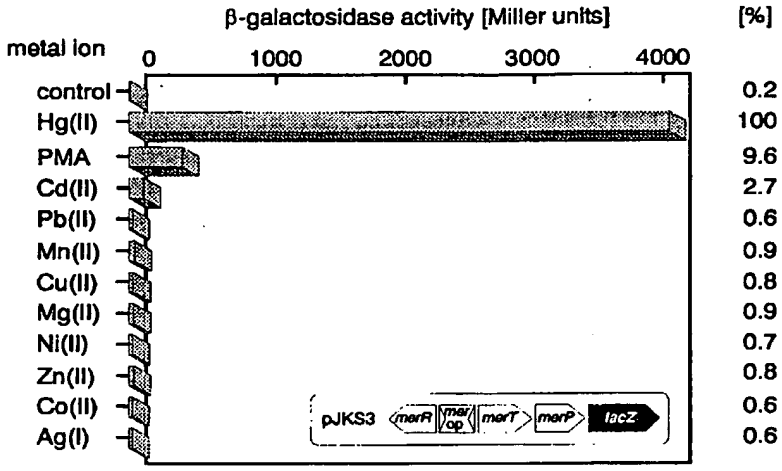


Figure 6. Specificity of the biosensor *E. coli* (pJKS3). Cultures were induced as described in materials and methods except 1 h induction time. The concentration of Hg(II) and PMA was 2×10^{-6} M, whereas the concentrations of the other metals were 10 times higher (2×10^{-5} M). The β-gal activity induced by Hg(II) (4169 Miller Units) was set to 100% and used as reference value.

The biosensor is specific for Hg(II)

A useful biosensor for Hg requires a high sensitivity as well as a high specificity towards the compounds to be monitored. *E. coli* JM109 (pJKS3) was incubated with different metal ions and phenylmercury acetate (PMA) and the correspondig β-gal activities were measured. $HgCl_2$, PMA, $CdSO_4$, $Pb(CH_3COO)_2$, $MnCl_2$, $Mg(CHCOO)_2$, $NiSO_4$, $ZnSO_4$, $CoCl_2$ and $AgNO_3$ were used in the test. According to the manufacturers' analyses, contaminations with other heavy metal ions, especially Hg(II), were negligible (0.002 to 0.005 %). Figure 6 summarises the results. The specificity was mainly restricted to Hg(II). Although the Tn*501* system is known to confer the narrow spectrum resistance, the organomercurial compound PMA induce β-gal synthesis, but only at a very high concentration. This might be due to Hg(II) contaminations within the organic compound. Of the heavy metal ions, only Cd(II) slightly interfered with the Hg(II)-specific system *in vivo*. This is in agreement with *in vitro* transcription assays using purified MerR (Ralston and O'Halloran, 1990). It interacted with MerR *in vivo* at ten times higher concentrations than Hg(II) and caused a forty times less transcriptional activation. In contrast to the *in vitro* results, Zn(II) and Ag(I) did not effect our biosensor E. Coli; JM109 (pJKS3).

Constitutively synthesised mer transport proteins considerably lower the time of β-gal induction by Hg(II)

Hg(II) ions have to enter the bacterial cell to induce transcription of the *mer* genes. Since the transport genes have to be induced first to import the inducer effectively, consitutively synthesised *mer* transport proteins were expected to decrease the time of induction. A 993 bp fragment from Tn*501* which encodes *merTP* and additionally contains the *mer* promoter and operator was inserted downstream of the *tetA* promoter of pACYC184 to give rise to pJKS13. The *tetA* promoter (*ptetA*) is a weak, constitutive promoter which should provide moderate transcription of *merTP*. To prove this assumption β-gal was translationally fused to the N-terminus of MerT (plasmid pJKS81, Tab. 1). As expected, the transcription signals of the *tetA* promoter and the translation initiation signals of MerT led to an elevated β-gal production (about 4000 Miller units). The plasmid pJKS13 was transformed into *E. coli* JM109 (pJKS3). The resulting strain JM109 (pJKS3, pJKS13) was incubated with Hg(II) for different times and the β-gal activities were measured. As illustrated in Figure 5B, the strain exhibited higher β-gal amounts after shorter induction times compared to the original strain JM109 (pJKS3). Constitutive expression of *merT* and *merP* seem to provide an improved Hg(II) uptake, which resulted in accelerated and higher β-gal production. Induction of the strain JM109 (pJKS3, pJKS13) by 10^{-7} M Hg(II) (20 ppb) for 45 minutes was sufficient to produce nearly 90% of the maximal β-gal activity obtained after 120 minutes of induction. By using an induction time of 15 minutes, which is less than an *E. coli* generation time, the sensitivity of the biosensor was about 0.5 to 1 ppb (2.5 to 5×10^{-9} M). In comparison to the original strain JM109 (pJKS3) with a detection threshold of about 2 ppb after 60 minutes of induction, this means a two- to fourfold increase in sensitivity and an fourfold decrease in induction time.

Hg(II)-induced plasmid replication improves the signal-to-noise ratio

To further lower the threshold value of Hg detection, the difference between Hg(II)-induced β-gal activity and uninduced β-gal basal level, the so-called signal-to-noise ratio, had to be increased. To find out if a change in the plasmid copy number had an influence on this ratio the same *mer-lacZ* fusion as in pJKS3, was introduced into plasmid pUC19 (pJKS19, Tab. 1) which has a higher copy number than pKO4 (McKenney et al., 1981). This increase in the copy number (pJKS19) was accompanied by an increase in the appropriate Hg(II)-induced β-gal activities, but, additionally, by an increase in the basal level (Tab. 2, Fig. 5C). The signal-to-noise ratio was not influenced since basal and induced activities changed proportionally (Tab. 2). Thus this ratio could only be influenced by alteration of the reporter plasmid copy

Table 2. Signal-to-noise ratio fo *E. coli* JM109 strains carrying different reporter plasmids at 5×10^{-10} M (0.1 ppb) and 5×10^{-9} M (1 ppb) Hg(II). The helper plasmid pJKS13 is present in all strains.

Plasmid	β-gal Activity (Miller units)		Signal-to-noise ratio
	uninduced	induced	
0.1 ppb			
pJKS3	15	20	1.3
pJKS19	150	180	1.2
pJKS68	26	78	3
1 ppb			
pJKS3	15	75	5
pJKS19	150	700	4.7
pJKS68	26	500	19.2

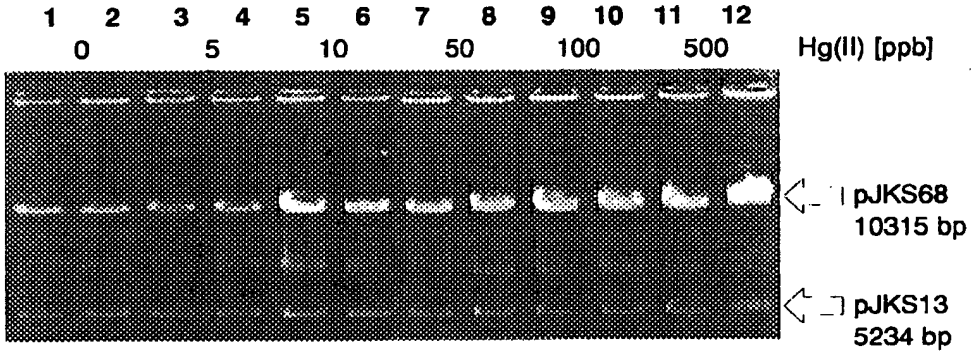


Figure 7. Hg(II)-dependent plasmid amplification of pJKS68. *E. coli* JM109 (pJKS68, pJKS13) was induced for 1.5 hours with different Hg(II) concentrations (in duplicate incubations). Plasmid DNA was isolated, cut with *Bam*HI and aliquots (according to 0.5 A_{600}) were loaded on a 1% agarose horizontal electrophoresis gel (lanes 1 to 12). The sizes of the linearised plasmids in base pairs are shown on the right.

number. We attempted hence a controlled increase of reporter plasmid copy number by Hg(II) MerR activated transcription. Therefore, an additional pMB1 origin of replication (*ori*) of pBR322 (Bolivar et al., 1977) missing its indigenous RNAII promoter was inserted as a 434 bp *Hae*III fragment at the 3′end of *lacZ* resulting in pJKS68. This additional *ori* is placed in pJKS68 downstream of the Hg(II)-inducible *mer* promoter, analogous to work of Panayotatos (1984) who used the *lac*UV5 promoter.

The new reporter plasmid pJKS68 and the *merTP* helper plasmid pJKS13 were cotransformed into *E. coli* JM109. As shown in Figure 5C, JM109 (pJKS68, pJKS13) produced comparable high β-gal amounts as JM109 (pJKS3, pJKS13) by induction with Hg(II). However, the basal enzyme activity in crude extracts was only 26 Miller units (Tab. 2), giving a sixfold decrease compared to JM109 (pJKS19, pJKS13). Using strain

JM109 pJKS68, pJKS13) the detection threshold could be decreased tenfold from 5×10^{-9} M (1 ppb) with JM109 (pJKS3, pJKS13) to 5×10^{-10} M (0.1 ppb) Hg(II). This is accompanied by a two- to threefold increase of the signal-to-noise ratio at 0.1 ppb (Tab. 2). Proof of Hg(II)-mediated plasmid amplification was given by incubating *E. coli* JM109 (pJKS68, pJKS13) with different Hg(II) concentrations and concomitant determination of the amount of reporter plasmid DNA. Whole plasmid DNA was purified by a quick lysis procedure (Sambrook et al., 1989). After digestion with *Bam*HI, identical aliquots of the linearised plasmids were loaded on a 1% horizontal agarose gel. As illustrated in Figure 7 the band intensity of the reporter plasmid appeared to expand proportionally to the increase in Hg(II) concentration whereas the signal of the linearised fragment of the reference plasmid did not change.

Detection of organomercurial compounds by using merB

The gene *merB* encodes the organomercurial lyase, which is able to cleave C-Hg bonds of organomercurial compounds. As seen in Figure 2 this gene is missing in Tn*501* but appears in other *mer* operons isolated from microorganisms displaying a so-called broad spectrum resistance. We speculated that this enzyme should release Hg(II) from organomercurials inside the biosensing bacterial cell and, by this way, induced β-gal synthesis. The promoterless *merB* gene was amplified from *Streptomyces lividans* (Sedlmeier and Altenbuchner, 1992) as a 655 bp *Bam*HI PCR fragment. This fragment was inserted into *Bam*HI-digested *merTP* helper plasmid pJKS13 downstrem of the *tetA* promoter and *merTP* to get pJOE2004. The *merTPB* helper plasmid pJOE2004 and the reporter plasmid pJKS68 were cotransformed into *E. coli* JM109 to verify the supposed induction response for Hg(II) and PMA. As shown in Figure 8B the strain appeared to produce β-gal in proportional relation to the added PMA concentration whereas JM109 (pJKS68, pJKS13), lacking *merB*, did not respond to organic PMA. The β-gal activity linearly increased up to a concentration of about 1.5×10^{-8} M. The detection threshold for PMA was 3×10^{-10} M PMA (0.1 ppb, data not shown). As a control, both strains were compared under induction with different Hg(II) concentrations and both showed significant and comparable β-gal production over the tested range, even at 5×10^{-10} M (Fig. 8A, Fig. 5C). Both strains produced β-gal in linear correlation to the added Hg(II) up to 6.25×10^{-8} M. In JM109 (pJKS68, pJOE2004) lower PMA concentrations led to a maximum β-gal response in comparison to Hg(II). This might probably be due to the respective intracellular availability of the inducer. Hg(II) is specifically transported into the cell via MerT and MerP whereas organic Hg compounds are supposed to enter the bacterial cell efficiently via passive diffusion.

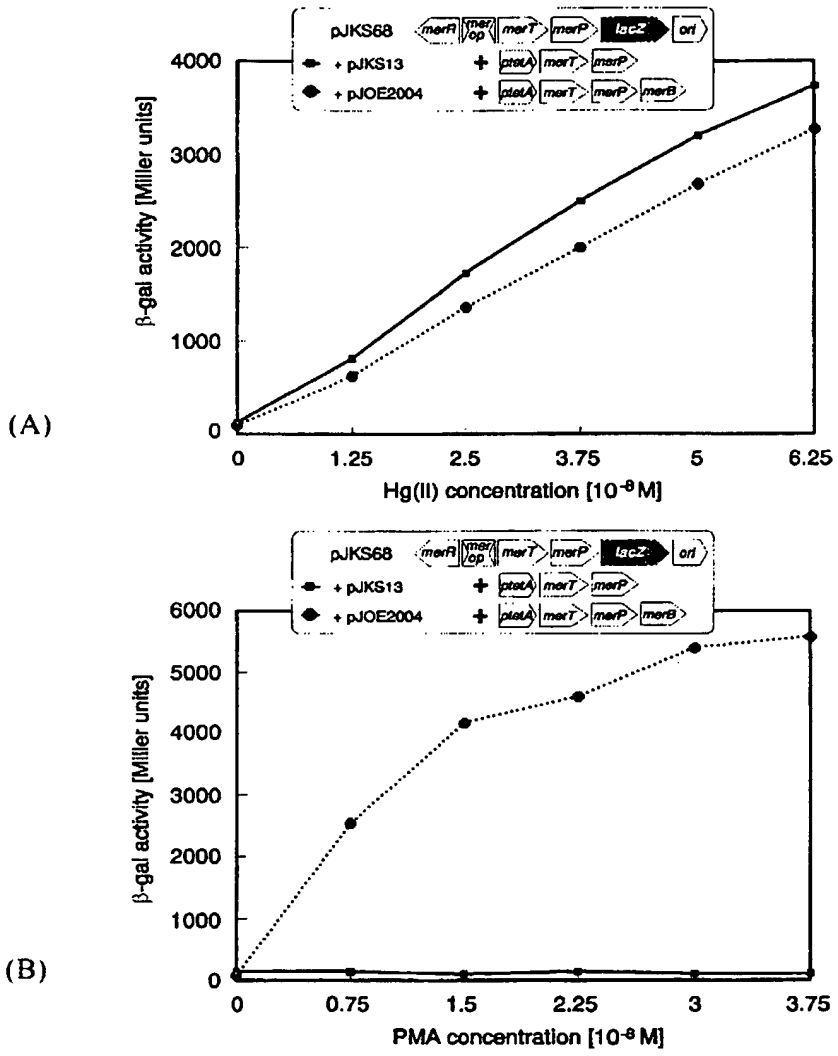


Figure 8. β-gal induction behaviour of *E. coli* JM109 (pJKS68, pJKS13) and JM109 (pJKS68, pJOE2004) at various concentrations of Hg(II) (A) and PMA (B) after 15 minutes induction time.

Development of an agar plate assay

A future perspective of the Hg(II) biosensor may be the experimental solution of a simple "Hg detection stick" which would resemble colour-based pH indicator sticks. Such a development may be the basis of a broad application of such biosensors. It should be possible to immobilise the bacterial cells on solid media or supports without affecting their inducibility. A promising step into this direction was achieved by the development of a Hg-specific plate assay on solid agar medium. In principle, this test is analogous to the histochemical detection of β-gal (Messer and Viel-

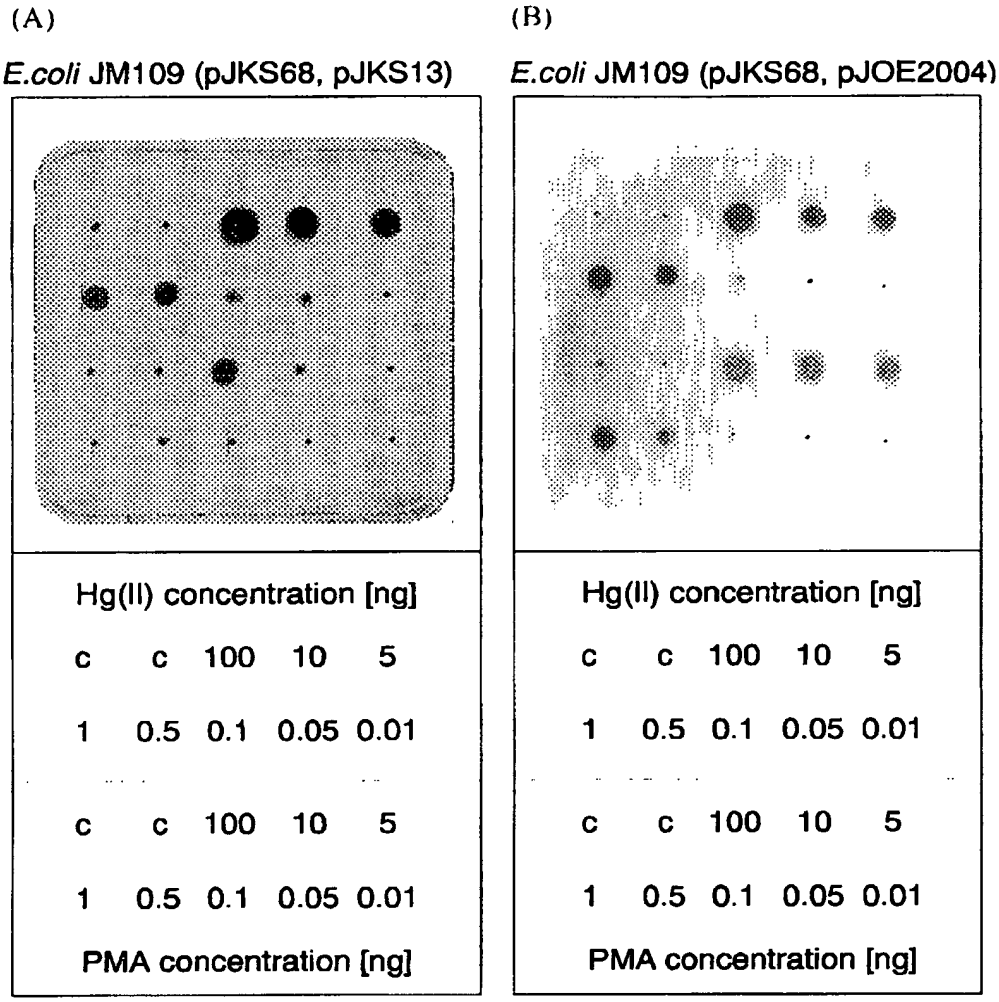


Figure 9. Agar plate assay with *E. coli* JM109 (pJKS68, pJKS13) (A) or JM109 (pJKS68, pJOE2004) (B). 10 µl of sterile Millipore water were used as a negative control (c).

metter, 1965). 100 µl of an overnight culture were spread on M9 minimal agar plates which were supplemented with 0.2% casamino acids, and the cells were grown for about 5 hours at 37°C. Serial dilutions of Hg-solutions were spotted in a 10-µl volume, and the plates were incubated for further 30 minutes at 37°C. Hg-dependent production of β-gal was detected by a soft agar overlay which contained 1 mg/ml X-gal as β-gal substrate. After 30 minutes of incubation at room temperature, blue colour development indicated β-gal activity and thus added Hg which appeared as Hg concentration dependent. A minimum of 0.1 ng Hg(II) or PMA in a 10-µl test volume (5×10^{-8} M, 3×10^{-8} M) could be detected, as seen in Figure 9.

Discussion

Transcriptional fusions of the regulatory elements of the Tn*501* specified Hg resistance and *lacZ* were used for the development of a sensitive microbial based Hg sensor. The use of LacZ as a reporter enzyme offered the possibility to rapidly analyse the signal by eye, by spectrophotometrical measurement or by chemiluminescence. Using AMPGD [3-(2′-spiroadamantane)-4-methoxy-4-(3′-b-D-galactopyranosyl-oxy)-phenyl-1,2-dioxetane] (Galacto-Light assay, Tropix, Bedford) and a luminometer, very low amounts of β-gal can be detected. With our microbial biosensor, the number of *E. coli* cells per assay could be reduced from about 10^9 cells with β-ONPG to 10^6 cells with AMPGD as substrate (data not shown). But with AMPGD the same concentration of Hg(II) was necessary to induce expression of *lacZ* and to significantly increase the β-gal activity above noninduced levels. Regarding the need of instrumental equipment, luminometer, and the cost of substrate AMPGD, the chromogenic assays may be preferable.

In the original construction, *lacZ* was fused with the N-terminal region of *merA* downstream of *merRopTP* which inactivated the Hg reductase gene. It was concluded that a functional Hg reductase would lower the inducer concentration by reduction of Hg(II). Indeed, if a functional Hg reductase, provided by the Hg resistance transposon Tn*21* on the plasmid pACYC184 (Tab. 1, De la Cruz and Grinstedt, 1982) was introduced into JM109 (pJKS3), about a tenfold higher concentration of $HgCl_2$ was needed to induce a detectable β-gal activity (data not shown).

Two other changes caused a dramatic improvement in the sensitivity of the assay and the time needed to perform *lacZ* induction. The first one was the addition of constitutively synthesised transport proteins MerT and MerP. The importance of the Mer transport proteins in activation of the *mer* operon transcription was shown before (Lund and Brown, 1987; Nakahara et al., 1979). Hg(II)-independent, constitutively produced Mer transport proteins, provided by a helper plasmid, reduced the time needed for Hg(II)-mediated induction of *lacZ* in pJKS3 from more than 120 min for JM109 (pJKS3) to 45 min for JM109 (pJKS3, pJKS13). In addition, the concentration of $HgCl_2$ needed for significant β-gal induction was reduced from about 1×10^{-8} M after 60 minutes of induction for JM109 (pJKS3) to 5×10^{-9} M after 15 minutes induction time using JM109 (pJKS3, pJKS13). The second increase in sensitivity from 1 to 0.1 ppb Hg(II) resulted from coupling the reporter plasmid replication and the transcription of the *mer* promoter. The reporter plasmid pJKS68 carries two replication origins. The original pMB1 origin of replication provides the normal pKO4 replication and the second, *mer* promoter dependent origin of replication leads to a Hg(II) inducible plasmid replication. As illustrated in Figure 7 the copy number increased about three- to fourfold at 10 ppb (5×10^{-8} M) and tenfold at 500 ppb (2.5×10^{-5} M). Expression of *lacZ* was enhanced by the

induction of transcription and the concomitant amplification in the copy number of the reporter plasmid.

A unique feature of the microbial biosensor presented in this paper is the expansion of its specificity from inorganic Hg(II) to organomercurials like PMA by providing the organomercurial lyase gene (*merB*) from the broad spectrum resistance of *Streptomyces lividans* on a helper plasmid. The specific activity of MerB in cells carrying the plasmid pJOE2004 was not determined, however, it is high enough to ascertain PMA concentrations with a comparable sensitivity (3×10^{-10} M) as $HgCl_2$ (5×10^{-10} M).

The maximal sensitivity of the described biosensor is about 3 to 5×10^{-10} M for a specific Hg compound after 15 minutes Hg(II) induction. Tescione and Belfort (1993) and Condee and Summers (1992) constructed *lux*-based reporter plasmid bearing *E. coli* strains. They described significantly higher detection thresholds of 2×10^{-8} M (30 min induction) or 1×10^{-8} M (2 to 3 minutes induction). The need for *mer* specific transport proteins to lower the detection threshold was reported by Selifonova et al. (1993) who described a bioluminescent sensor *E. coli* strain using fusions of Tn*21 mer* genes to the *Vibrio fisheri luxCDABE* operon. In contrast to their reported similar sensitivity (5×10^{-10} M) after 40 minutes induction time they described a very slow growth of the sensor strain with only an increase of 0.2 units in the optical density in 24 hours combined with the need for an adaptational growth over three days before the start of the Hg test procedure. In contrast to this poor growth performance our sensor strain constructs show normal cultivational behaviour. From this it is obvious that the lowest detection threshold with the minimal growth and induction time is performed by the strains described in this report. They provide a practical approach to differentiate organic from inorganic Hg compounds and measure the important bioavailable Hg which is not tightly bound to macromolecules in soil or sewage (Selifonova et al., 1993). By using these two biosensors, which recognise inorganic and organic Hg, it would be possible to differentiate Hg contaminations. Risk assessment could be facilitated focusing on organomercurial contaminations, which are much more toxic than Hg(II). The investigations reported here focused on Hg salt solutions, because only rare data are available referring to the behaviour of Hg compounds in samples containing high amounts of organic substances. Too little is known about the mobility and the availability of biologically active Hg in complex organic material. Using the bacterial biosensors, it may be possible to shed more light on these important questions.

Besides Hg, regarded as the most toxic heavy metal, microorganisms developed a variety of resistance mechanisms against other heavy metals such as Cr(VI) or Ni(II) (Silver et al., 1989; Silver and Walderhaug, 1992). If the transcription of these resistance genes is specifically regulated, the same experimental solutions as described in this report may be used to provide other heavy metal biosensors with altered specificity.

Instead of these *in vivo* assays, it may also be possible to make use of the specific binding of a heavy metal to the regulatory protein of the appropriate resistance system for the development of an advanced *in vitro* test. The Hg-sensing protein component of the Hg resistance system is the regulatory protein MerR. It has a nanomolar sensitivity ($K_D < 10^{-8}$ mol × l^{-1}) and is highly selective for Hg (Ralston and O'Halloran, 1990). The affinity constant of MerR for Hg(II) ($K > 10^8$ l× mol^{-1}) is comparable to the affinity (association) constant of some antibodies for their antigen ($K = 10^8$ l 10^{11} l× mol^{-1}). These properties of MerR make this protein the best candidate as sensor component in the development of a Hg-specific *in vitro* assay. Such a test could be developed as sandwich test similar to the conventional enzyme-linked immuno sorbant assay (ELISA) using antibody enzyme conjugates for signal production. MerR should be coupled to a reporter enzyme, similar to an antibody-enzyme conjugate, which may be tested by gene fusion of *merR* to a reporter gene of interest.

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A highly sensitive and specific assay using a novel human growth hormone cDNA reporter gene regulated by the human interleukin-4 inducible germline ε transcript promoter

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Abstract

We have successfully developed a highly sensitive and specific assay system for human interleukin-4 (IL-4) regulated gene expression. It is based on a human Jijoye cell line with the germline ε transcript promoter joined to the human growth hormone (hGH) cDNA. The germline ε transcript promoter is responsive to IL-4 and involved in immunoglobulin heavy chain class switching. We cloned hGH complementary DNA (cDNA) as the reporter gene instead of using conventional hGH genomic DNA which failed to generate any IL-4 inducible clone in human Jijoye cells. The two IL-4 inducible cell lines with the hGH cDNA reporter show high signal/noise ratio for IL-4-mediated induction (60–90 fold). The response to IL-4 is dose-dependent with ED_{50} of 10 pM. As expected, there is no response to other human cytokines and growth factors, as well as mouse IL-4. The mutant hIL-4 antagonist hIL-4.Y124D inhibits the induction mediated by native hIL-4. These IL-4 inducible cell lines provide a sensitive, specific assay system to study IL-4-regulated gene expression, and in particular the regulation of the germline ε promoter.

Keywords: Human interleukin-4; Human growth hormone cDNA; Reporter gene; Germline ε transcript promoter

1. Introduction

Human interleukin-4 (IL-4) and IL-13 are major cytokines responsible for B cell immunoglobulin class switching to IgE (Lebman and Coffman, 1988; Pene et al., 1988; Gascan et al., 1991; de Vries et al., 1991; Punnonen et al., 1993; Ezernieks et al., 1996). Prior to class switching, IL-4 has been found to induce a germline ε transcript initiated upstream of the switch region (Rothman et al., 1990; Gauchat et al., 1990; Rothman et al., 1991). This transcript is apparently not translated into protein, but its expression is required for IgE production. Transient transfection of the germline ε transcript promoter linked to a luciferase gene into a Burkitt's lymphoma BL2 cell line showed a 2–3 fold induction of the luciferase gene expression by human IL-4 (Cocks et al., submitted).

Abbreviations: EPO, erythropoietin; GM-CSF, granulocyte macrophage colony stimulating factor; hGH, human growth hormone; IFN, interferon; IL, interleukin; PCR, polymerase chain reaction; TGF, transforming growth factor; TNF, tumor necrosis factor

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Our goal was to obtain a stable cell line that incorporated the IL-4 inducible germline ε promoter into a simple, rapid assay format. Such a cell line could be used for high through-put assays in pharmaceutical research and development. The creation of such cell lines depends on the stable integration of the selected reporter gene into the genome of cytokine-responsive host cells at random chromosomal positions. Since the varying chromosomal contexts of the integrated reporter gene have different effect on expression and regulation in different host cell lines (Feinstain et al., 1982), it is desirable to obtain a random integration into the host genome. A second consideration for stable cell lines to be used for screening assays is the ease of measurement of the reporter gene product. Human growth hormone (hGH) is particularly good because it is secreted into the medium and can be measured sensitively by ELISA or RIA; no cell disruption is necessary. Vectors based on hGH genomic DNA have been developed and are widely used in the studies of transient gene expression (Selden et al., 1986).

In the present study, we have constructed hGH reporter plasmids containing the germline ε transcript promoter and have stably transfected them into human Jijoye cells. Two highly IL-4 inducible clones were obtained when a hGH cDNA was used; none were obtained with the more commonly used genomic hGH DNA. We discuss reasons for this difference and present a characterization of the two IL-4 inducible clones.

2. Materials and methods

2.1. Cell cultures

The human Jijoye cell line (ATCC CCL87) was cultured in normal growth medium: RPMI 1640 medium supplemented with 15% horse serum (donor herd, from Sigma, St. Louis, MO, USA), 5% heat-inactivated fetal bovine serum (Life Technologies, Gaitherburg, MD, USA), 2 mM L-glutamine, 50 μg/ml Streptomycin and 100 IU/ml Penicillin (Life Technologies). G418-resistant stable clones were selected and maintained in normal growth medium adjusted to 1 mg/ml or 500 μg/ml G418 (Life Technologies), respectively. Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2.

2.2. Human growth hormone cDNA cloning

The human growth hormone (hGH) cDNA was cloned using the strategy of reverse transcription followed by PCR. The poly(A) + RNA was isolated from a stable Jijoye clone constitutively expressing a high level of hGH (Jenh et al., unpublished results) following the recommendation of a commercial QuickPrep mRNA purification kit (Pharmacia, Piscataway, NJ, USA). An aliquot of RNA was primed with a specific 3′ end primer for the first strand cDNA synthesis using Molony Murine Leukemia Virus RNaseH$^-$ Reverse Transcriptase (M-MLV H$^-$ RT [Superscript], Life Technologies) according to the manufacturer's recommendation. Isolated mRNA amounting to 4 μl was mixed with 50 pmol of a 3′ end primer (B2380) and distilled water was added to a final volume of 12 μl. The mixture was heated to 70°C for 10 min and then quickly chilled on ice. The contents of the tube was collected by brief centrifugation and 4 μl of 5 × Reaction Buffer (1 × reaction buffer contains 50 mM Tris–HCl, pH 8.3, 75 mM KCl and 3 mM $MgCl_2$), 2 μl of 0.1 M DTT and 1 μl of mixed dNTP stock (10 mM each dATP, dGTP, dCTP and dTTP at neutral pH) were added to the tube. The solution was mixed by gentle vortexing, collected by brief centrifugation and placed at 37°C for 2 min to equilibrate the temperature. Finally, 1 μl (200 U) of M-MLV H$^-$ RT was added, mixed gently and incubated at 37°C for 1 h. An aliquot of the RT reaction was then used as the template for PCR with a specific 5′ end primer (B2379) and the 3′ end primer B2380. The oligonucleotide primers (B2379 and B2380) used for the RT and PCR are shown below and contain incorporated restriction sites (*Bam*HI and *Eco*RI) to facilitate the cloning. B2379: 5′-TTCGGATCCCAAGGCCCAACTCCC-3′; B2380: 5′TTCGAATTCAGTCAGACAAAATGATGCAACTTAATTTTATTAGGACAAGGC-3′. The 823 bp PCR product containing the authentic hGH cDNA was digested with *Bam*HI and *Eco*RI, and then gel-purified. After subcloning into a cloning vector pSP72 (Promega,

Madison, WI, USA), the hGH cDNA was verified by both restriction analysis and DNA sequencing.

2.3. Plasmid construction of pGεhGHcDNA

The 600 bp DNA fragment containing the germline ε transcript promoter was removed from plasmid 933-24 as a *Hin*dIII and *Bcl*I. It was subcloned from a genomic λ clone CH4AHIgE12, which spans the constant and ε switch regions (Nishida et al., 1982). The detailed DNA sequence information for the germline ε transcript promoter has been published (Mills et al., 1990). It is located within the Ig switch region Sε between *Bam*HI and *Bcl*I restriction sites. The ligation of three DNA fragments containing the germline ε transcript promoter (*Hin*dIII/*Bcl*I), the hGH cDNA (*Bam*HI/*Eco*RI) and the cloning vector pSP72 (*Hin*dIII/*Eco*RI), respectively was carried out. The final reporter plasmid, used for the stable transfection into Jijoye cells, was designated pGεhGHcDNA.

2.4. Stable transfection and G418 selection in Jijoye cells

The human Jijoye cells were freshly split 16 to 24 h before electroporation and seeded at a density of 5×10^5 cell/ml. The following day the Jijoye cells were collected by centrifugation (at 1000 rpm for 3–5 min), counted and resuspended in normal growth medium at a density of 2×10^7 cell/ml. 5×10^6 Jijoye cells in 250 μl were placed in a disposable electrophoretic cuvette (4 mm) and were preincubated with 10 μg of pGεhGHcDNA and 1 μg of pRSVNEO (ATCC 37198) at room temperature for 10 min. The cells were then subjected to a voltage pulse of 200 V at a capacitance of 960 μF using a Gene Pulser apparatus with capacitance Extender (BioRad, Rockville Center, NY, USA). Following a recovery period of 10 min at room temperature, electroporated Jijoye cells were gently dispersed in 10 ml of normal growth medium in a T25 flask. Forty-eight hours post-electroporation, the cells were centrifuged as described above and resuspended in 80 ml of selection medium containing 1 mg/ml of G418. Approximately 1.2×10^4 cells in 200 μl were seeded per well into four 96-well plates. The wells containing individual G418-resistant clones were picked and expanded into 24-well plates for the screening using hGH ELISA assay.

2.5. ELISA assay of human growth hormone

For screening G418-resistant Jijoye stable clones, the expression level of human growth hormone (hGH) was assayed by ELISA. After expanding individual G418-resistant stable clones, the same number of cells for each stable clone were seeded into 96-well microtiter plates with different concentrations of human IL-4 which was derived from IL-4-producing Chinese Hamster Ovary (CHO) cells with a biological activity of 10^7 U/mg (Schering-Plough Research, Kenilworth, NJ, USA). In one experiment, a neutralizing monoclonal antibody, 25D2 (Schering-Plough Research) (Chretien et al., 1989), against human IL-4 was used to demonstrate the specificity of IL-4-mediated induction. After 72 h incubation with IL-4, unless otherwise indicated, the medium supernatants were analyzed by hGH ELISA. Briefly, flat-bottomed Nunc immuno-plates were coated with sheep anti-hGH (Biodesign International, Kennebunkport, ME, USA) diluted 1:2000 in 50 mM carbonate–bicarbonate buffer (pH 9.5). After incubation for overnight at 4°C, the plates were then incubated for 90 min at room temperature with BLOTTO [5% non-fat dry milk and 0.05% Tween 20 in Dulbecco's Phosphate Buffered Saline (DPBS)] to saturate protein binding sites. The plates were washed with 10 mM potassium phosphate buffer (pH 7.4) containing 0.05% Tween 20. Medium supernatants or hGH standard (Boehringer Mannheim, Indianapolis, IN, USA) were then added to the plates and incubated for 90 min at room temperature. The plates were washed and the rabbit anti-hGH (DAKO, Carpinteria, CA, USA) diluted 1:1000 in assay buffer [0.25% BSA, 0.05% Tween 20 and 1% sheep serum (Sigma) in DPBS] was added and incubated for 90 min at room temperature. The plates were then washed and incubated for 1 h at room temperature with goat anti-rabbit IgG peroxidase (Boehringer Mannheim) diluted 1:10,000 in assay buffer. After washing, ABTS enzyme substrate (Boehringer Mannheim) was added, incubated for 25 min at room temperature and read on a ELISA reader for the absorbance at 405 nm.

For all ELISA results, O.D. 405 was converted into ng/ml of hGH based on the standard curve.

3. Results

3.1. Background and rationale

The germline ε transcript promoter used in this project was reported to be the *cis* element for the IL-4-mediated induction of the germline ε transcript and to be involved in inducing human B cells to switch to IgE-producing cells by IL-4 (Rothman et al., 1990; Gauchat et al., 1990; Rothman et al., 1991). We initially used the human growth hormone (hGH) genomic DNA as a reporter gene linked to the germline ε transcript promoter to construct IL-4-responsive plasmids. Upon transient transfection into Jijoye cells, this construct showed about 2–3 fold IL-4-mediated induction. This DNA was then stably transfected into human Jijoye cells. Among the 27 stable clones screened, three clones constitutively expressed high levels of hGH with no significant IL-4-mediated induction and the remainder showed low signal and no IL-4-mediated induction. In two other stable transfections of human Jijoye cells using the hGH genomic DNA linked to the CD23 promoter, similar results were observed. Thus, we were unable to generate stable cell lines containing an IL-4 inducible germline ε construct using a hGH genomic reporter gene after three stable transfections.

We examined the hGH genomic DNA and found two interesting DNA sequences in the hGH genomic DNA not mentioned in the originally published sequences (DeNoto et al., 1981). The first was a glucocorticoid receptor binding site in the first intron (Moore et al., 1985) and the second was an Alu repetitive element (274 bp) downstream of the 3′ untranslated region (Seeburg, 1982). The Alu repetitive element has been reported to be a potential 'hot spot' for recombination in some genes (Vnencak-Jones and Phillips, 1990). It may result in an unfavorable chromosomal context by not allowing the integration of the reporter gene at random chromosomal positions and therefore, lowering the probability of obtaining an IL-4 inducible cell line. To improve the hGH reporter gene and avoid the regulatory and Alu DNA sequences in the genomic DNA, we cloned a hGH cDNA. This is the first example we know of using hGH cDNA as the reporter gene.

3.2. Plasmid construction and transient expression

The specific 600 bp DNA fragment containing the germline ε transcript promoter was linked to a hGH cDNA clone as described in Section 2. The restriction mapping of the final reporter plasmid (designated pGεhGHcDNA) is shown in Fig. 1. This plasmid was electroporated into Jijoye cells for transient expression and the medium supernatants were assayed by hGH ELISA 48 h post-transfection. The result shows that both the cloned cDNA and genomic hGH constructs responded to human IL-4 in a dose-dependent manner with 2–3 fold induction (data not shown).

3.3. Screening of IL-4 inducible stable Jijoye clones

The plasmid pGεhGHcDNA was cotransfected with pRSVNEO into Jijoye cells for stable expression and the electroporated cells were plated into

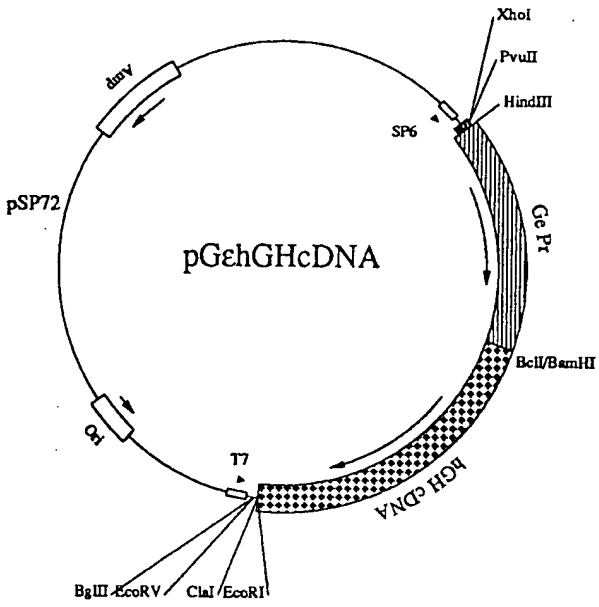

Fig. 1. Restriction mapping of the plasmid pGεhGHcDNA. The human growth hormone cDNA (hGH cDNA) is shown between *Bam*HI and *Eco*RI restriction sites. The germline ε transcript promoter (Ge Pr) is shown between restriction sites for *Hin*dIII and *Bcl*I. After the ligation, both *Bam*HI and *Bcl*I were not regenerated (*Bcl*I/*Bam*HI). The plasmid backbone is from pSP72.

96-well plates for G418 selection. The G418-resistant stable clones selected in 96-well plates were picked and further expanded into 24-well plates with 1 ml medium. Screening was done in 96-well plates using small cell samples directly from the 24-well plates. The results of the preliminary screen of 30 stable clones were encouraging. In addition to three clones constitutively expressing high levels of hGH with low IL-4 induction and two slightly IL-4 inducible clones with low basal levels, two highly IL-4 inducible stable clones, C5 and C12, were identified. The pattern of stable cell lines with hGH cDNA reporter gene is what might be expected when the reporter gene is integrated at random chromosomal positions. Since in our hands no IL-4 inducible clones were observed using the hGH genomic DNA as a reporter gene, the use of the hGH cDNA appeared to give a significant advantage which may be useful with other promoter elements, particularly in human cell lines.

3.4. Characterization of IL-4 inducible clones

To demonstrate the specificity of the IL-4-mediated induction in clones C5 and C12, a neutralizing monoclonal antibody, 25D2, against human IL-4 was coincubated with IL-4. The result shows that these two IL-4 inducible clones responded to human IL-4 in a dose-dependent manner; moreover, the monoclonal antibody 25D2 completely blocked the induction of hGH expression by human IL-4, indicating the induction of clones C5 and C12 is mediated by human IL-4.

The kinetics of the IL-4-mediated induction of the germline ε promoter was examined in clone C5. Fig. 2 shows that the IL-4-mediated induction of hGH expression increased with the time up to 96 h with significant induction occurring between 24 and 48 h incubation. Based on this result, 72 h IL-4 incubation was chosen for subsequent experiments. Similar results were obtained with clone C12. The kinetics of IL-4-mediated induction of reporter gene expression observed here was consistent with that of germline ε RNA induction (Gauchat et al., 1990).

The signal/noise ratio and the fold of IL-4-mediated induction for both C5 and C12 were examined. The results in Fig. 3 indicate that the C12 clone was slightly superior to the C5 clone due to a lower basal level in the absence of IL-4 and a slightly higher signal after the IL-4-mediated induction. Fig. 3 also

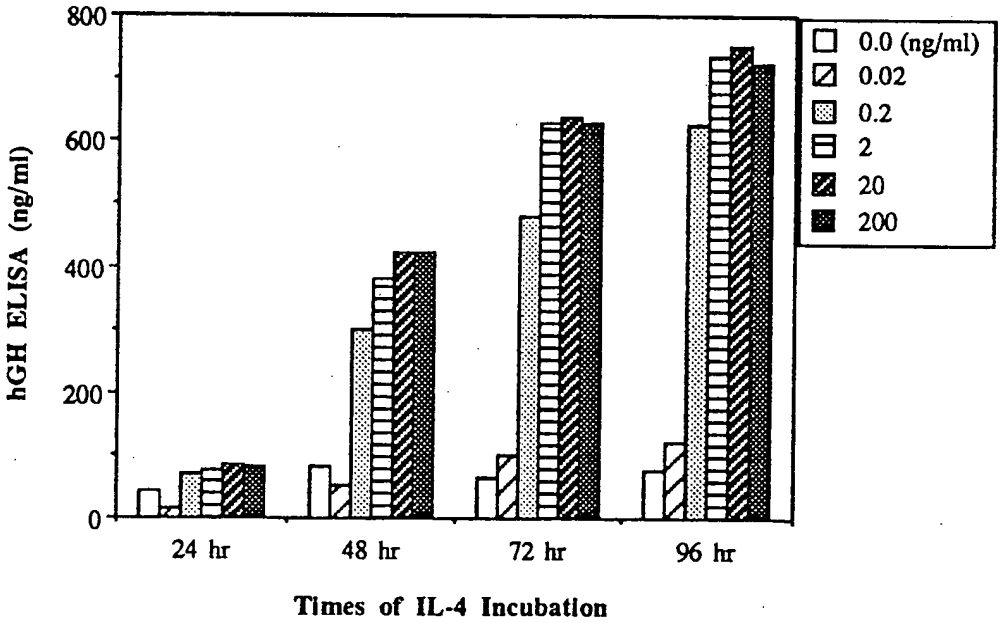

Fig. 2. Time course of the IL-4-mediated induction. The C5 stable clone was seeded in a 96-well plate at 37,500 cells per well and incubated with human IL-4. At different times indicated, 100 μl medium supernatants were assayed for hGH expression by ELISA as described in Section 2.

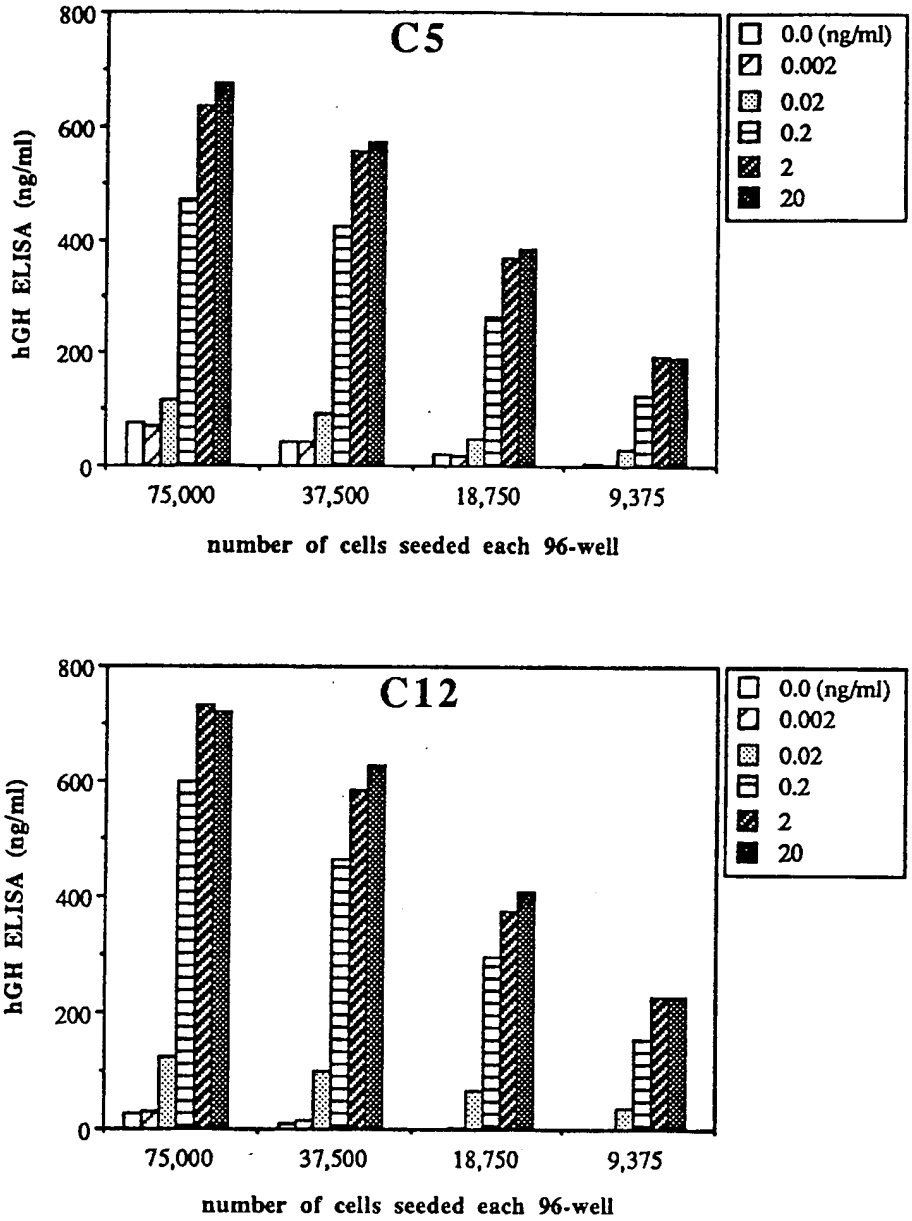

Fig. 3. The signal/noise ratio of IL-4-mediated induction. Both C5 and C12 clones were seeded in 96-well plates with the indicated numbers of cells in each well and incubated with human IL-4. After 72 h of IL-4 incubation, the medium supernatant from each well was assayed by hGH ELISA. The top graph is for the C5 clone and the bottom one for the C12 clone.

indicates that about 40,000 cells (or fewer) per assay point were sufficient to generate a signal/noise ratio > 10 for IL-4-mediated induction after 72 h incubation. The IL-4-mediated induction of the C12 clone was 60–90 fold and therefore, clone C12 was chosen for further examination based on this result. To test the consistency of the C12 clone, assays were set up randomly over a 96-well plate either with or without IL-4. The result indicates that the standard deviation of IL-4-mediated induction was $< 5\%$ ($n = 30$). The response of clone C12 as a function of IL-4 concentration was examined and the ED_{50} of induction is

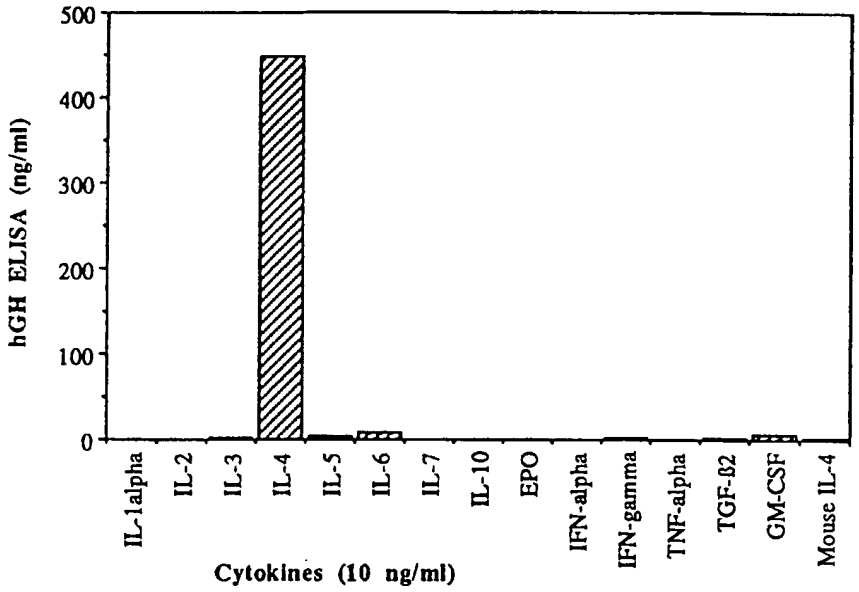

Fig. 4. The cytokine specificity of the IL-4-mediated induction. The C12 cells were incubated with a variety of human cytokines or growth factors at 10 ng/ml. Mouse IL-4 was also included. After 72 h of incubation, the medium supernatants were assayed by hGH ELISA.

10 pM (data not shown). It is consistent with dissociation constant Kd of 2–100 pM for human IL-4 receptor binding (Zurawski et al., 1993).

3.5. Effect of other cytokines

To examine the specificity of hIL-4 inducible hGH expression, C12 cells were incubated with a variety of human cytokines or growth factors at 10 ng/ml, including IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, EPO, IFN-α, IFN-γ, TNF-α, TGF-β2 and GM-CSF. The results (Fig. 4) indicated that only human IL-4 induced hGH expression. In addition, mouse IL-4 at 10 ng/ml did not induce hGH expression, consistent with its species specificity. Furthermore, C12 cells were also tested with

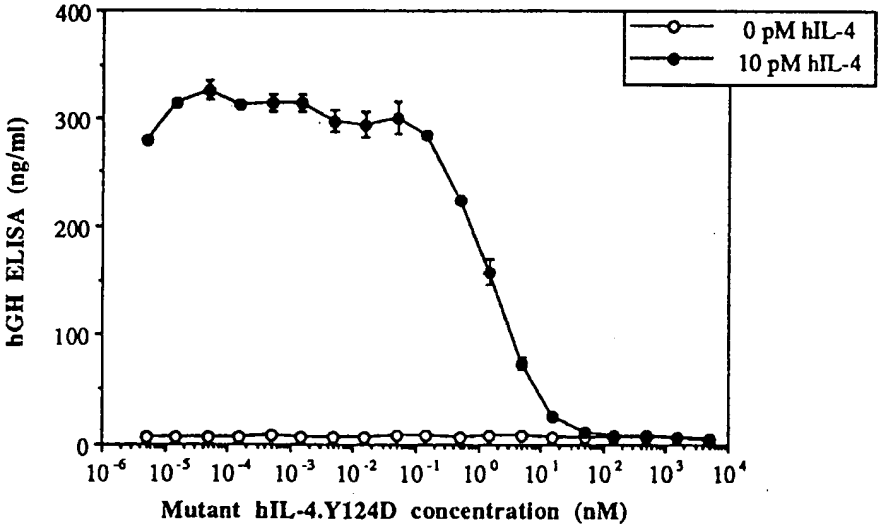

Fig. 5. Inhibition of IL-4-mediated induction by mutant hIL-4.Y124D. The C12 cells were incubated with various concentrations of hIL-4.Y124D in the absence or presence of native hIL-4 (10 pM). After 72 h of incubation, the medium supernatants were assayed by hGH ELISA.

hIL-13 and the result shows that hIL-13 was unable to induce any hGH expression at concentration up to 2 μM (data not shown).

3.6. Inhibition of IL-4-mediated induction by mutant hIL-4 antagonist

The effect of hIL-4.Y124D, a competitive antagonist of the biological activities of native hIL-4 (Kruse et al., 1992; Zurawski et al., 1993), on IL-4-induced hGH expression in C12 cells was investigated. C12 cells were incubated with various concentrations of hIL-4.Y124D in the absence or presence of suboptimal concentration (10 pM) of native hIL-4. Fig. 5 shows that hIL-4.Y124D inhibited the IL-4-mediated induction of hGH expression in C12 cells in a dose-dependent manner. Inhibition of IL-4-induced hGH expression by 50% was observed at about 2 nM. However, hIL-4.Y124D, even at a concentration of up to 5000 nM, was unable to induce any hGH expression in C12 cells.

4. Discussion

The generation of stable cell lines containing a human growth hormone reporter gene regulated by specific responsive DNA elements provide not only sensitive and specific assays, but also provide high through-put screens for antagonists/agonists of selected transcription inducers. Human growth hormone (hGH) is desirable relative to other reporter genes, such as chloramphenicol acetyltransferase, β-galactosidase and luciferase because no cell disruption is necessary. A vector based on hGH genomic DNA has been developed and widely used in the studies of transient gene expression (Selden et al., 1986). To our knowledge, hGH genomic DNA has not been used to obtain human stable cell lines, although a stable Vero (monkey) cell line responsive to type I interferon was established using the genomic clone (Lleonart et al., 1990). In fact, our repeated early attempts with hGH genomic DNA linked to either CD23 promoter or germline ε transcript promoter failed to identify any IL-4 inducible stable Jijoye cell lines. Nonetheless, we were able to obtain such IL-4 inducible cell lines using β-galactosidase as a reporter gene (Cocks et al., submitted). A careful examination of hGH genomic DNA identified an Alu repetitive element (274 bp) downstream of the 3′ untranslated region, which may direct the reporter gene integration into unfavorable chromosomal context or 'hot spots' for recombination and thus, fail to generate any IL-4 inducible cell line. Indeed, when we used a hGH cDNA as a reporter gene, two highly IL-4 inducible stable Jijoye clones (C5 and C12) were identified from screening of 30 stable clones. This hGH cDNA should prove very useful for any further reporter construct with other inducible DNA elements or promoters.

Induction of hGH expression regulated by the germline ε transcript promoter in Jijoye cells is a specific property of IL-4, since none of other cytokines or growth factors tested were effective. In addition, no cross-species activity was observed since mouse IL-4 had no effect. Recently, human IL-13 has been demonstrated to induce IgG4 and IgE synthesis and CD23 expression by human B cells (Punnonen et al., 1993). However, hIL-13 did not have any effect on our stable Jijoye cell lines using either hGH cDNA or β-galactosidase as a reporter with the germline ε promoter; it has been proposed that the EBV positive Burkitt lymphoma Jijoye does not express hIL-13 receptors (Cocks et al., submitted).

These IL-4 inducible cell lines reported in this paper represent a simple and specific means to screen for IL-4 antagonists/agonists. The observation that mutant hIL-4 antagonist hIL-4.Y124D at a low nM level efficiently inhibits the hGH expression induced by native hIL-4 in C12 cells indicates that this screen system is capable to identify potential antagonists/agonists specific for hIL-4.

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Comparison of mutant forms of the green fluorescent protein as expression markers in Chinese hamster ovary (CHO) and *Saccharomyces cerevisiae* cells

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Abstract

Several green fluorescent protein (Gfp) mutants with increased cellular fluorescence compared to the wildtype protein have recently been generated. We have expressed and compared wildtype Gfp and mutants S65T, F100S/M154T/V164A, F64L/S65T, and S65A/V68L/S72A under identical growth conditions in CHO and *Saccharomyces cerevisiae* cells. The results suggest that the last two Gfp mutants are the best candidates as reporter proteins, and they provide a high signal-to-noise ratio in both systems. Single gene copy expression of these mutant forms is easily detectable over background autofluorescence. All Gfps are highly stable within cells, with an estimated 1/2-life between 7 h (wildtype) and 70 h (F100S/M154T/V164A) in *S. cerevisiae* cells. Although this limits their use in examining rapid cellular events without further modification, Gfp is expected to be a useful marker for monitoring the physiological state of cells in bioreactors using on-line probes.

Keywords: Green fluorescent protein; Chinese hamster ovary; *Saccharomyces cerevisiae*

1. Introduction

Reporter proteins are commonly used in biotechnology to study various aspects of the gene expression machinery and thereby improve gene expression, as markers for following cell lineages, and for the screening of cell populations. Choice of the reporter protein is often dictated by ease of protein expression in the cell system and by the

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ability to detect and quantitate protein concentrations at a high signal-to-noise ratio without the need for multiple preparatory steps. Additionally, there is growing appreciation of the fact that any cell population is inherently heterogeneous, necessitating single-cell analyses. Non-invasive, single-cell detection is thus a pre-requisite if the reporter protein is to be used for identifying, isolating, and re-culturing desired population subsets.

The green fluorescent protein from *Aequorea victoria* satisfies most of these requirements, as is evident from the growing number of reports on using Gfp for monitoring gene expression non-invasively in living cells and organisms (Cubitt et al., 1995). However, there are some drawbacks associated with wild type Gfp, such as a time lag in the detection of the fluorescent form of Gfp due to the requirement of a post-translational, oxygen dependent modification step in the fluorophore formation, a lower detection sensitivity than most other enzymatic reporter systems, thermosensitivity of protein folding, and a maximal excitation peak in the UV region of the electromagnetic spectrum (Heim et al., 1994, 1995; Inouye and Tsuji, 1994; Lim et al., 1995; Ogawa et al., 1995). The wildtype form is hence not suitable for detecting low levels of gene expression or fast changes in expression levels.

As an approach to overcome these drawbacks, several mutant versions of Gfp have been created using DNA shuffling (Crameri et al., 1996), chromophore directed mutagenesis (Heim et al., 1994, 1995; Delagrave et al., 1995; Cormack et al., 1996), or random mutagenesis (Ehrig et al., 1995; Anderson et al., 1996; Siemering et al., 1996; Kimata et al., 1997), and selecting for a brighter, green-fluorescing phenotype (Table 1). These mutations are tentatively classifiable into two groups. One group of mutations alter the chromophore or its surrounding environment, resulting in altered (red-shifted) excitation spectra and increased molar extinction coefficients without altering the emission spectrum or the quantum yield. The other group decreases the temperature sensitivity of the apoprotein to folding correctly, resulting in increased sensitivity of detection. Several mutants possessing both properties have also been reported. Most of these mutant forms have been evaluated in various cell systems, under different growth conditions, with varying modes and levels of protein expression, and using different assays. A comparative study under similar conditions has not been reported. Moreover, the effects of various mutations on the stability of the reporter protein have not been examined. This is of particular importance, since the 1/2-life of the protein affects the rate at which maximal or steady-state levels of expression are attained (Thompson et al., 1991). Monitoring cell-cycle or circadian phenomena require reporters with excellent time resolution capabilities, and increased protein stability is disadvantageous, although one could observe the rate of change of level of a stable reporter protein rather than the absolute changes in reporter protein levels. Sensitivity of the reporter protein should hence be increased without compromising requirements of speed of response.

In this work, we have expressed and compared various Gfp mutants under similar conditions in CHO and *S. cerevisiae* cells to determine their brightness relative to wildtype Gfp. Additionally, in order to determine whether Gfp is a suitable reporter for observing rapid induction kinetics or cell-cycle associated protein synthesis, we have estimated the intracellular stability of Gfp in *S. cerevisiae* cells using a technique that does not use antibiotics. The results of this study may be useful in determining the mutant that is best suited as a marker for a given application.

2. Materials and methods

2.1. Cell lines and growth conditions

Chinese Hamster Ovary (CHO) cells obtained from Dr David A. Bernlohr (Department of Biochemistry, University of Minnesota) were maintained and grown as previously described (Subramanian and Srienc, 1996).

S. cerevisiae YPH399 (*MATa*, *ade*2-101, *leu*2Δ1, *lys*2-80, *his*3Δ200, *trp*1Δ63, *ura*3-52) was obtained from Dr Bodley (Department of Biochemistry, University of Minnesota Medical School). Absence of uracil in the growth medium was used to select for all plasmids used in this

Table 1
Reported properties of Gfp mutants

Mutant	Excitation emission	Relative brightness	Solubility	Fluorophore formation (h^{-1})	Comments	Reference
Wildtype	395 (470)/508 (503)	1	Low			Chalfie et al., 1994
				0.3465	In *E. coli*	Heim et al., 1995
				0.438	In *E. coli* at 37°C	Crameri et al., 1996
				7.05	In yeast at 37°C	Siemering et al., 1996
I167T	471 (396)/502 (507)	1.88	ND	ND	In *E. coli*, fluorimetry	Heim et al., 1994
E222G	481/506	ND	ND	ND	In *E. coli*	Ehrig et al., 1995
F64M/S65G/Q69L	490/505	ND	ND	ND	In *E. coli*	Delagrave et al., 1995
S65T	489/511	6	ND	1.54	In *E. coli*, fluorimetry	Heim et al., 1995
			Low	0.49	In vitro, pseudo first-order rate	Reid and Flynn, 1997
					Thermosensitive at 37°C	Kimata et al., 1997
F64L/S65T (**FM1**)	488/507	35	>90%	ND	In *E. coli*, fluorimetry	Cormack et al., 1996
		4[b]			In 293T cells, flow cytometry	Yang et al., 1996
S65A/V68L/S72A (**FM2**)	481/507	29	Completely soluble	ND	In *E. coli*, fluorimetry	Cormack et al., 1996
F100S/M154T/V164A[c] (**CYC3**)	396 (471)/508	45	High	0.438	In *E. coli*, fluorimetry	Crameri et al., 1996
		42			In CHO cells, fluorimetry	
		46			In CHO cells, flow cytometry	
S65T/V163A	488/507	16	ND	ND	In NIH3T3 cells, flow cytometry	Anderson et al., 1996
V163A/S175G	396 (471)/507	7.7	High		In *E. coli* at 37°C, fluorimetry	Siemering et al., 1996
				2.3	In yeast at 37°C, fluorimetry	
S65T/V163A/S175G/I167T	ND	32.5	ND	ND	In Cos-7 cells, transient expression, flow cytometry	Siemering et al., 1996

Table 1 (Continued)

Mutant	Excitation/emission	Relative brightness	Solubility	Fluorophore formation (h^{-1})	Comments	Reference
V163A/S175G/I167T	396 (471)/507[a]	19.6	ND	ND	In Cos-7 cells, transient expression, flow cytometry	Siemering et al., 1996
S65T/S147P	496/512	3[b]	ND	ND	In *E. coli* at 37°C, fluorimetry	Kimata et al., 1997
		5.14[b]			In mouse L-cells, microscopy	

ND, not determined.
Values in parentheses indicate secondary excitation or emission peaks except [a], where both excitation peaks have the same magnitude.
[b] Relative to S65T mutant, transient gene expression.
[c] Contains an Ala residue insertion after the fMet.

Table 2
Mammalian expression vectors

Plasmid	Cloning strategy	Promoter
pCMVβ		Human cytomegalovirus immediate early promoter; negative control
pRc/CMV/wt	*Hin*dIII-*Xba*I digested and cloned into pRc/CMV	Human cytomegalovirus immediate early promoter
pRc/CMV/S65T	Excised cDNA with *Bam*HI, blunt ended and cloned into blunt ended *Hin*dIII site of pRc/CMV	Human cytomegalovirus immediate early promoter
pSRα/CYC3		SRα hybrid promoter
pSRα/FM1	*Xba*I-*Eco*RV digested and cloned into pSRα/CYC3	SRα hybrid promoter
pSRα/FM2	*Xba*I-*Eco*RV digested and cloned into pSRα/CYC3	SRα hybrid promoter

All enzymes were purchased from Life Technologies or New England Biolabs (Beverly, MA) and used according to the manufacturer's recommendations.

study (*URA3* marker). Cells were grown at 30°C on SD minimal medium supplemented with adenine sulfate (100 mg l^{-1}), histidine (80 mg l^{-1}), lysine (150 mg l^{-1}), leucine (50 mg l^{-1}), and tryptophan (20 mg l^{-1}). Galactose or glucose (2% w/v) were used as the carbon source.

2.2. Expression vectors

The cDNAs encoding the wild type and S65T mutant green fluorescent proteins contained on plasmids pBSK – /Gfp and pRSET/S65T were kindly provided by Dr R.Y. Tsien of the Howard Hughes Medical Institute at the University of California, San Diego (Heim et al., 1994). A shuffled mutant, CYC3, (F100S, M154T, V164A) of the *gfp* cDNA was obtained in a mammalian expression vector, pSRα, from Dr W.P.C. Stemmer at the Affymax Research Institute (Palo Alto, CA; Crameri et al., 1996). The Gfp cDNA for the mutants FM1 (F64L, S65T) and FM2 (S65A, V68L, S72A) were obtained from Dr Stanley Falkow at Department of Microbiology and Immunology, Stanford University School of Medicine (Cormack et al., 1996). pRc/CMV was obtained from Invitrogen Corp. (San Diego, CA), and pCMVβ encoding *Escherichia coli* β-galactosidase was purchased from Clontech (Palo Alto, CA). The expression vectors used are shown in Table 2.

The parent plasmid pRS169 used in the studies with *S. cerevisiae* was provided by Dr Hieter (Johns Hopkins University, Baltimore). pSEY18/GAL1-10/GFP, a wildtype Gfp yeast expression vector, was obtained from Dr R.Y. Tsien. The galactose inducible GAL1 promoter was used to drive Gfp synthesis in all expression vectors (Johnston et al., 1994). Details of the expression vectors constructed are provided in Fig. 1. Standard molecular biology protocols were followed during the construction of all expression vectors (Sambrook et al., 1989). Large quantities of transfection quality DNA were prepared using Plasmid Maxi Kit (Qiagen, Chatsworth, CA) or Biggest Prep Kit (5 Prime- > 3 Prime Inc., Boulder, CO). DNA was quantitated by agarose gel electrophoresis using calibration standards (Gibco/BRL) and spectrophotometry (absorbance at 260 nm).

2.3. Transfection

CHO cells were transfected by lipofection with various expression vectors as previously described (Subramanian and Srienc, 1996).

Yeast cells were transformed as previously described (Soni et al., 1993).

2.4. Fixation

The use of alcohols such as ethanol or methanol as the fixative have previously been reported to completely bleach wildtype Gfp fluorescence (Chalfie et al., 1994). We have compared three fixatives for use with immunofluorescence of *S. cerevisiae* cells expressing Gfp (Table

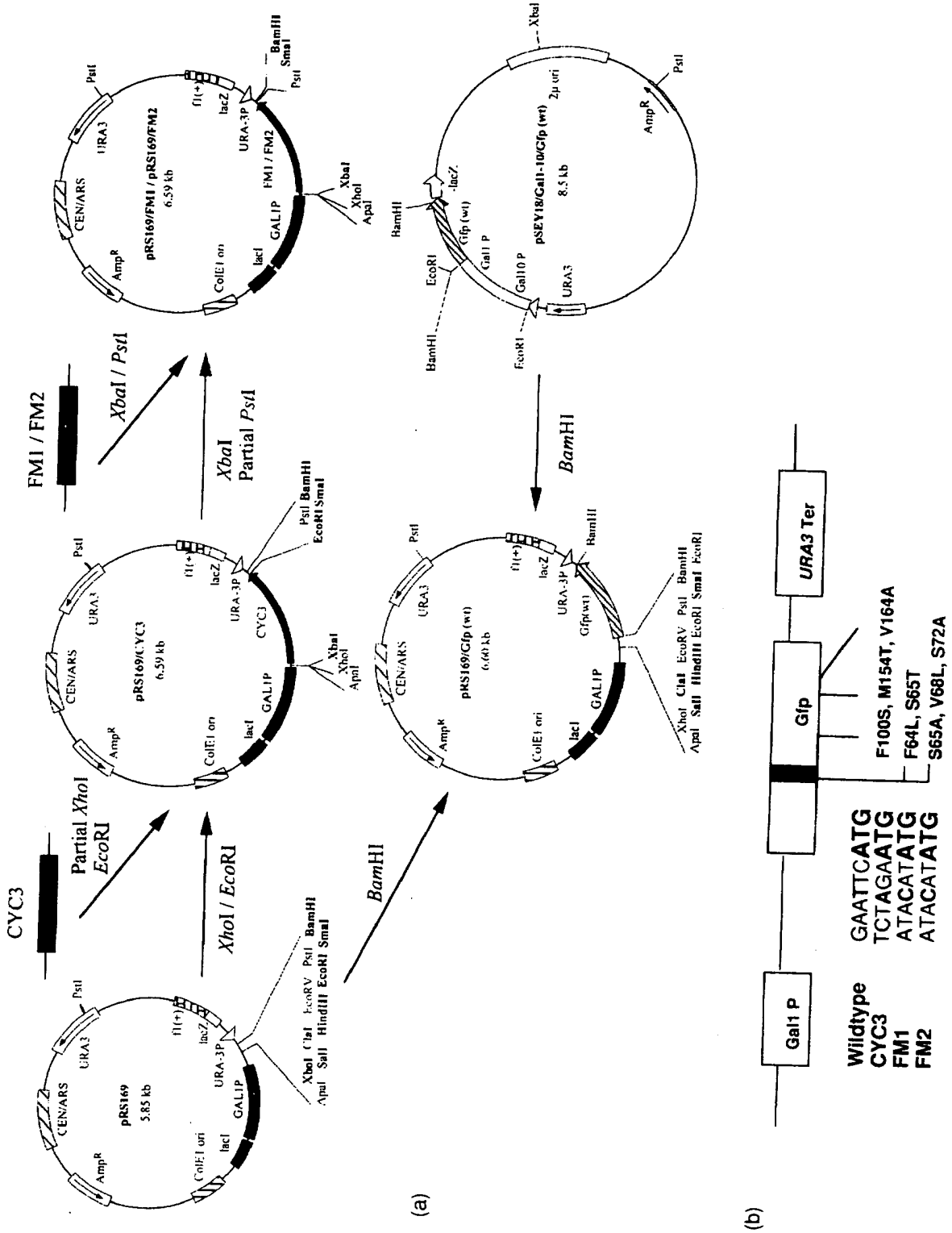
CYC3
Partial XhoI EcoRI
XhoI / EcoRI
BamHI
FM1 / FM2
XbaI / PstI
XbaI Partial PstI
BamHI
pRS169 5.85 kb
pRS169/CYC3 6.59 kb
pRS169/FM1 / pRS169/FM2 6.59 kb
pRS169/Gfp (wt) 6.60 kb
pSEY18/Gal1-10/Gfp (wt) 8.5 kb
(a)
(b)
Gal1 P
Gfp
URA3 Ter
Wildtype GAATTCATG
CYC3 TCTAGAATG
FM1 ATACATATG
FM2 ATACATATG
F100S, M154T, V164A
F64L, S65T
S65A, V68L, S72A

Fig. 1.

3), and methanol-free formaldehyde was chosen as the fixative. Fixation decreased mean fluorescence, but did not alter the shape of distribution of fluorescence intensity observed in the population. CHO cells were trypsinized and fixed as previously described (Subramanian and Srienc, 1996).

2.5. Immunofluorescence staining

S. cerevisiae cells expressing Gfp from a 2μ *ori*-plasmid were stained for immunofluorescence analysis, and a titer analysis for the primary polyclonal rabbit anti-Gfp antibody (Clontech, Palo Alto) concentration (1:200, 1:100, 1:50) revealed that even the lowest concentration of antibody was saturating at a cell concentration of 10^7 cells per ml. Manufacturer's recommendations were followed for the titer of the secondary antibody (*R*-phycoerythrin (PE)-conjugated goat anti-rabbit IgG $(F(ab'))_2$ fragment, Sigma Immunochemicals, St. Louis). A linear correlation between green fluorescence intensity and immunofluorescence was observed after accounting for spectral overlap. A monoclonal murine anti-Gfp antibody (Clontech) and PE-conjugated goat anti-mouse IgG secondary antibody (Sigma) combination was tested for both CHO and *S. cerevisiae* cells; however, no immunofluorescence was observed (Fig. 2d). Unstained Gfp expressing cells and cells stained for another antigen with PE-conjugated antibodies were used to compensate for emission spectral overlap between PE and Gfp. Unstained cells not expressing Gfp were used as a control to distinguish between autofluorescent cells and cells expressing Gfp. Fixed CHO cells were stained for intracellular Gfp content as described previously (Subramanian and Srienc, 1996).

2.6. Protein separation and detection

CHO cells were trypsinized 36 h post transfection and pelleted at 400 g for 12 min. The cell pellet (10^6 cells) was washed with 1 ml PBS, resuspended in 50 μl sample loading buffer (Sambrook et al., 1989) containing 5% (v/v) β-mercaptoethanol, and boiled at 100°C for 5 min. Samples were run with molecular weight standards (Kaleidographic, Bio-Rad Laboratories, Hercules) and recombinant Gfp standards (Clontech) on precast ready polyacrylamide gels (Tris-glycine, 12.0%, Bio-Rad) as per manufacturer's recommendations. The proteins were subsequently transferred to a 0.2 μm polyvinylidene difluoride (PVDF) membrane (Bio-Rad). The membrane was blocked with Tris-buffered saline containing 3% bovine serum albumin (TBS-BSA) for 1 h. The membrane was then washed and incubated in TBS-BSA containing Rabbit anti-Gfp antibodies at 1:2000 dilution for 1 h. All subsequent steps were performed using the Immunoblot-SAP kit for rabbit primary antibody according to the manufacturer's recommendations (Zymed Laboratories, San Francisco). The western blot was quantitated using a densitometer (Molecular Dynamics) and ImageQuant data analysis software.

2.7. Flow cytometry

Flow cytometry was carried out on an Ortho Cytofluorograf IIs (Ortho Diagnostics, Westwood, MA) as previously described (Subramanian and Srienc, 1996). In order to maximize Gfp specific fluorescence and minimize interference from autofluorescence, different excitation wavelengths (457 and 488 nm) and emission filters combinations (525 ± 25 BP, 515 LP and 495 LP, Ortho Diagnostics) were tested. Autofluorescence was found to be higher using 457 nm for excitation. The ratio of mean Gfp fluorescence/

Fig. 1. Strategies used to construct various centromeric plasmids expressing different Gfps. (a). The plasmids constructed had identical selection markers, origins of replication, and promoter and termination sequences in the expression cassette. Unique sites in the multiple cloning site are highlighted. (b) The plasmids differed in the sequences immediately upstream of the translation start codon. The untranslated upstream sequence is dispensable in yeast, and the identity of the base at the -3 position with respect to the translation start codon (shown in bold) alters the level of expression less than 2-fold. Amino acid substitution mutations in Gfp sequence corresponding to various mutants are also shown.

Table 3
Effect of fixatives on Gfp fluorescence

Fixative	Wildtype	Flourescence intensity			
		CYC3	FM1	FM2	Mean ± S.D.
No fixative	100	100	100	100	
Ethanol[a]	7.20	51.80	27.60	27.14	28.43 ± 18.3
Formaldehyde (methanol free)[b]	47.07	82.05	69.30	35.8	56.55 ± 20.9
Commercial formalin[c]	85.81	117.95	42.03	31.25	69.26 ± 40.1

The ratio of the fluorescence observed post-fixation to the native fluorescence of unfixed cells is expressed as a percentage. The results are thus normalized for the inherent differences in fluorescence intensities of different Gfps and are hence directly comparable.

[a,b,c] Choice of fixative affects the CVs of DNA distributions obtained in cell cycle studies. *S. cerevisiae* cells expressing Gfp were fixed in ice-cold 70% ethanol (Dien et al., 1994) or commercial formaldehyde (Eitzman and Srienc, 1991) as previously described. For paraformaldehyde fixation, cells were spun down and re-suspended in freshly prepared 0.25% methanol-free formaldehyde. Following a 5 min incubation at room temperature, cells were spun down and fixed in ice-cold 70% ethanol. Use of ethanol as a fixative yielded best DNA staining results.

autofluorescence was used as an index to measure the separation between autofluorescent and fluorescent cells, and excitation at 488 nm and collection using the FITC bandpass filter gave the highest ratio for all Gfps. A 630 nm long pass filter (Rolyn Optics, Covina, CA) was used to determine red fluorescence (PE) intensity. Three parameters, forward angle light scatter (FALS), right angle light scatter (RALS), and green fluorescence intensity were acquired in list mode. Green fluorescence was acquired using both linear and logarithmic modes. For immunologically stained cells, red fluorescence was acquired in place of RALS in either the linear or log mode. Light scattering data was used to gate out debris for all samples. Software compensation of list mode data (Cyclops data analysis system) was used to account for the spectral overlap between Gfp and PE emission spectra by using appropriate controls.

2.8. Confocal microscopy

Confocal images were collected on a BioRad MRC1024 confocal microscope using a × 100 objective at a resolution of 512 × 512 pixels. Pinhole size (4.0) and electronic zoom factor (3.0) were optimized for collecting images of yeast cells. Gfp and PE fluorochromes were excited using the 488 and the 568 nm laser lines respectively, and FITC and Texas Red filter sets were used to collect green and red fluorescence. A sequential analysis of 10 images at a step size of 0.75 μm was performed.

2.9. Flow cytometric data analysis

The region of autofluorescence was determined using the 'threshold method' (Sladek and Jacobberger, 1993) by defining a gate to include 98% of cells in the control population, and using the gate to distinguish between autofluorescent and Gfp expressing cells. The mean fluorescence of the expressing and the autofluorescent cells were then computed.

3. Results

3.1. Immunofluorescent staining and co-localization analysis

Wildtype Gfp fluorescence in yeast cells grown at 30°C or higher has been reported to be weak (Lim et al., 1995). It has been hypothesized that a large fraction of Gfp molecules are improperly folded at these elevated temperatures, resulting in decreased fluorescence. In our experiments, we have not experienced any difficulty in detecting wildtype Gfp expression in *S. cerevisiae* cells

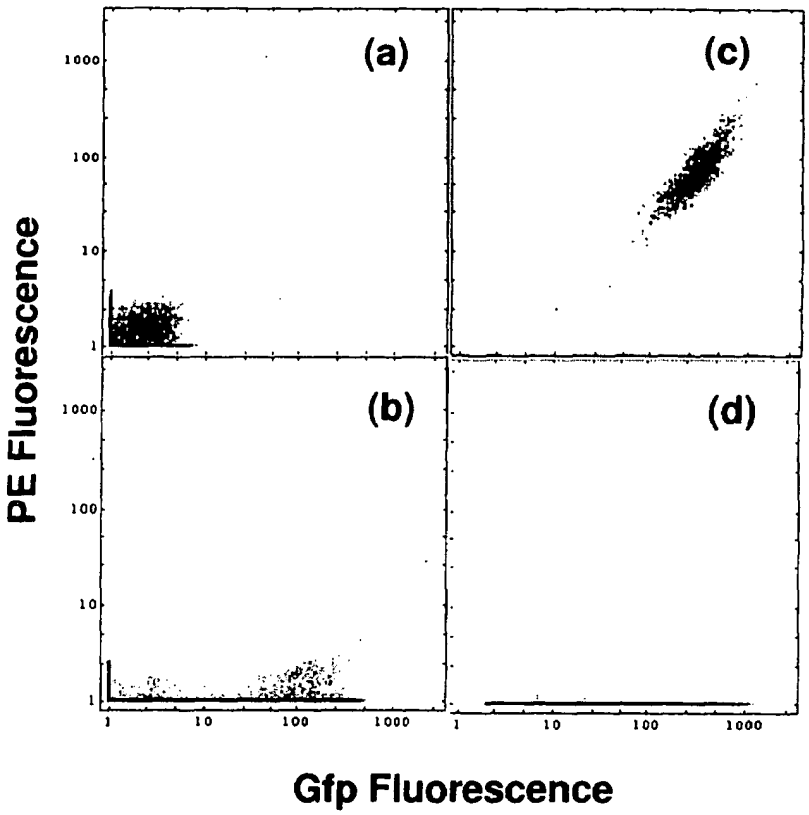

Fig. 2. *S. cerevisiae* cells expressing wildtype Gfp from a multi-copy (2 μ) plasmid (c) and negative control samples not expressing Gfp (a) have been stained with fluorescent antibody for Gfp content. Intensity of Gfp fluorescence (indicative of fluorescent Gfp content) is linearly proportional to the intensity of *R*-PE fluorescence (indicative of total Gfp content) (c). Both Gfp and PE fluorescence signals have been compensated for spectral overlap. There was no non-specific staining by the secondary antibody (b). Staining with a monoclonal anti-Gfp antibody did not yield good results (d).

grown at 30°C, either by flow cytometry or by microscopy. In order to test whether our strains did indeed possess a significant non-fluorescent fraction of Gfp, we expressed wildtype Gfp from a multicopy (2μ *ori*) plasmid in *S. cerevisiae* YPH399 and detected Gfp content by inherent green fluorescence and by immunofluorescence staining. Cells expressing Gfp could easily be identified by comparing their green fluorescence intensity distributions with corresponding distributions of negative controls. Samples of cells not expressing Gfp stained with both the primary and secondary antibodies (Fig. 2a) and of Gfp expressing cells stained with only the secondary antibody (Fig. 2b) revealed that there was no non-specific binding of either antibody to *S. cerevisiae* cells. A pronounced linear correlation was observed between single cell green fluorescence (fluorescent Gfp content) and the corresponding immunofluorescence intensity (*R*-PE; total Gfp content) over a wide range of fluorescence values of cells expressing Gfp (Fig. 2c). This indicates that fluorescent Gfp content is a good measure of total intracellular Gfp content within the bounds of the amount of protein over-expressed in single yeast cells. Immunofluorescence staining of *S. cerevisiae* cells expressing Gfp from a single copy (CEN/ARS *ori*) plasmid could not be detected, presumably due to the low levels of antigen present.

Fluorescence microscopy of fixed cell samples revealed that fixation causes a re-distribution of intracellular protein content, as was previously observed in CHO cells (Subramanian and Srienc,

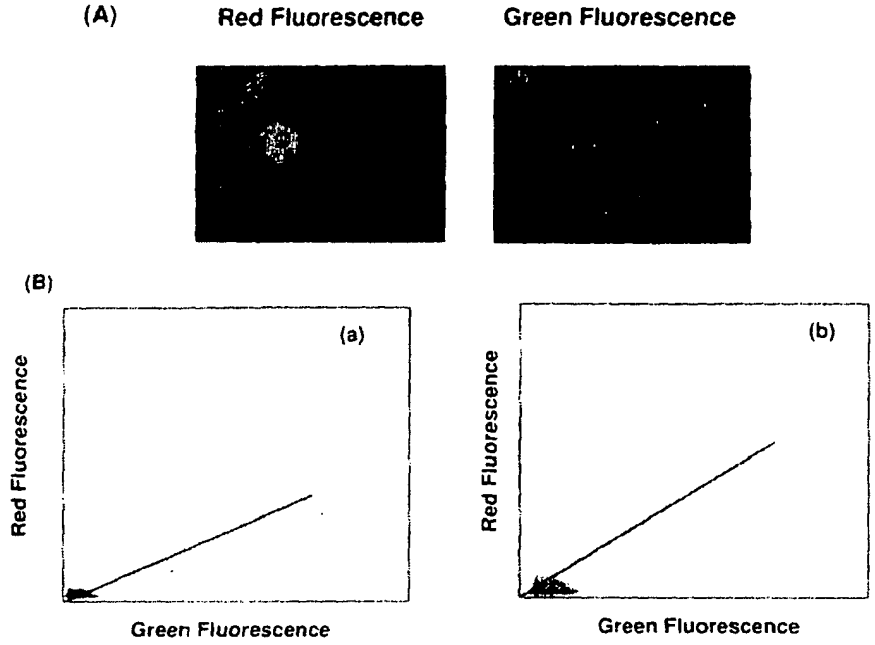

Fig. 3. (A) A 3-dimensional reconstruction of single yeast cells expressing wildtype Gfp from a multicopy (2 μ) plasmid and stained for intracellular Gfp content using a PE-conjugated antibody bridge. The green and red fluorescence images (representing fluorescent and total Gfp content) were obtained using different excitation wavelengths and emission filters to eliminate spectral overlap. (B) Co-localization analysis has been performed, and dot-plots of the number of voxels as a function of green and red fluorescence intensity (256 channels each) are shown. Immunofluorescence and green fluorescence are indeed co-localized, as is shown for two representative cells (a and b). The threshold for the background has been set at 10^6 voxels. Estimated linear regressions between green and red fluorescence are also provided.

1996). After fixation, intracellular fluorescence was observed to be diffuse throughout the cell with a few spots of punctate fluorescence (Fig. 3A). As an external control for the efficacy of the immunofluorescent staining protocol, stained cells were analyzed using a confocal microscope. A co-localization analysis of green fluorescence and immunofluorescence was performed to determine the fraction of non-fluorescent protein and its spatial distribution within cells. Scanning conditions were optimized to minimize photobleaching of both Gfp and PE fluorescence. A 3-dimensional reconstruction and superposition of scans obtained revealed that immunofluorescence and green fluorescence appear co-localized (Fig. 3B). No significant sites of immunofluorescence staining in the absence of Gfp fluorescence were observed, indicating that non-fluorescent Gfp forms a relatively small fraction of total Gfp content and is diffuse throughout the cell.

3.2. Relative brightness of mutants in CHO cells

CHO cells were identically transfected with plasmids encoding for various Gfps and analyzed by flow cytometry. Un-normalized green fluorescence intensities of cells harvested 36 h post-transfection were compared to determine relative brightness of Gfp mutants. All mutants were significantly brighter than the wildtype, with mutants FM1 and FM2 appearing the brightest (Table 4A). This analysis assumes that identical transfections result in identical protein expression levels. Since this is frequently not the case, it is necessary to normalize the fluorescence observed by the amount of protein present in cells. Variations in total intracellular Gfp content were estimated by immunofluorescence staining and western blot analysis using polyclonal antibodies raised against wildtype protein.

CHO cells transfected with plasmids encoding for β-galactosidase, wildtype Gfp, and mutants

Table 4
(A) Relative brightness of Gfp mutants in CHO cells and (B) relative brightness and 1/2-life of Gfp mutants in *S. cerevisiae*

Gfp	Brightness (unnormalized)	Normalized brightness	
		Immunofluorescence	Western blot
Wildtype	1	1	1
CYC3	4.8	3.2	4.4
FM1	6.7	ND	6.0
FM2	6.6	ND	14.2
S65T	2.1	2.6	2.2

Gfp	Relative brightness		
	Mean fluorescence	Threshold method	Degradation rate (h^{-1})
Wildtype	1	1	
CYC3	4.2	1.5	<0.01
FM1	127.5	21.6	0.02–0.05
FM2	100.9	21.1	0.02–0.03
Wildtype (2 μ)	8.7	3.5	0.10

Since the error associated with the Threshold method increases with increasing degree of overlap between positive and negative populations, these results provide an underestimate of the relative brightness of Gfp mutants, while a direct comparison of mean fluorescence values without normalizing for autofluorescence provides an over-estimate of relative brightness. Degradation rates were estimated either by ignoring the formation of new fluorescent Gfp molecules subsequent to termination of transcription (upper estimate), or by fitting experimental data to the structured model (Subramanian and Srienc, 1996).

S65T and CYC3 were stained with fluorescent antibodies against Gfp, and analyzed (Fig. 4). The positive correlation between the green fluorescence intensity (proportional to fluorescent Gfp content) and the immunofluorescence intensity (proportional to total Gfp content) of fluorescent single cells was used to compare Gfp mutants. However, a pronounced saturation in immunofluorescence intensity was observed for the CYC3 mutant, and to a lesser degree in cells expressing wildtype Gfp. Since increasing antibody titer did not alleviate this effect, it was likely caused by diffusional constraints to staining. The fact that the effect was pronounced for CYC3 expressing cells indicates that these cells produced the maximum amount of protein, and this was verified by Western blotting (see below). Immunofluorescence data in the unsaturated regime was fit to a linear function of green fluorescence to obtain an index of fluorescence per unit protein. The results (Table 4A) indicate that both CYC3 and S65T mutants are indeed brighter than wildtype Gfp.

Concerns in using immunofluorescence staining to detect intracellular protein are possible masking of available epitopes, steric hindrance, or diffusivity problems within cells due to the disparity in size between Gfp and the large secondary antibody. To account for such potential problems, a Western blot analysis was performed to detect denatured protein on a blot in the absence of diffusional constraints and to determine the average Gfp content per cell in absolute units. Gfp content of CHO cells transiently expressing wildtype Gfp, and mutants CYC3, S65T, FM1, and FM2 were determined (Fig. 5). All Gfps expressed had a similar size to the rGfp standards from *E. coli*, indicating that there was no post-translational modification of the protein in CHO cells. Although all transfections were carried out under identical conditions, cells expressed differing amounts of Gfp. The band corresponding to the S65T mutant is faint suggesting a lower expression level or transfection efficiency compared to the other mutants, or a lower affinity to the

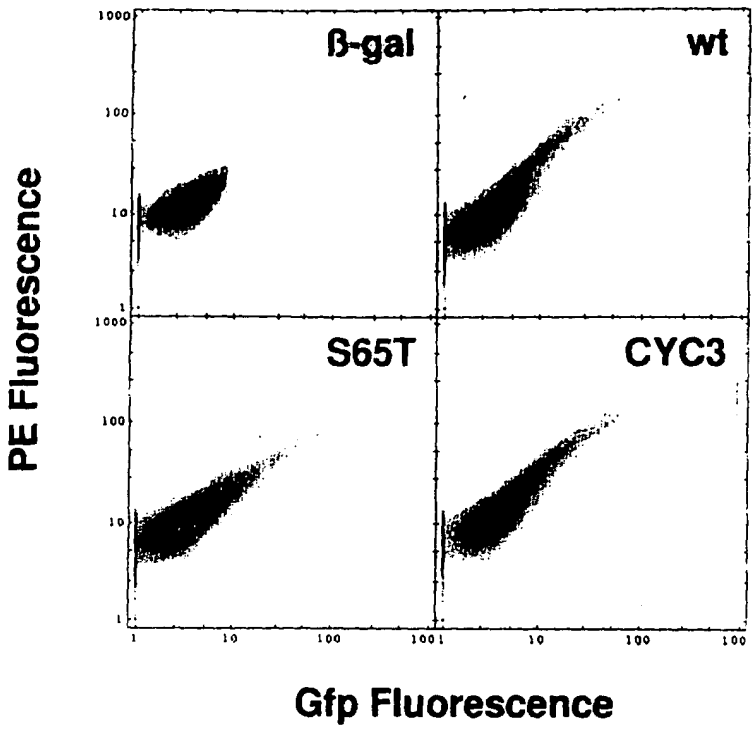

Fig. 4. CHO cells transiently expressing Gfps were stained with fluorescent (PE) antibody bridge for Gfp content. Immunofluorescence intensity (*R*-PE, proportional to total Gfp content) is positively correlated to green fluorescence intensity (Gfp, proportional to fluorescent Gfp content) for cells with lower intracellular Gfp concentration (channel numbers less than 50), but appears to saturate for cells containing larger amounts of Gfp. Data acquired logarithmically were linearized, fitted to linear regressions, and the slopes were compared to determine the relative brightness of Gfp mutants. Results from this analysis are given in Table 4A.

antibody. Expression was highest for the CYC3 mutant, followed by wildtype Gfp. Intensity of protein bands were quantitated by densitometry and a comparison of green fluorescence intensity

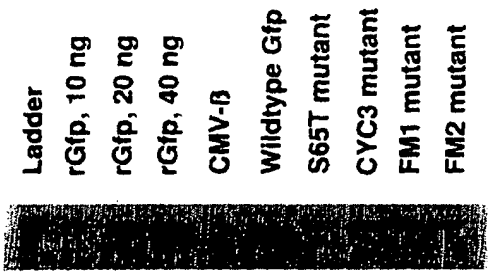

Fig. 5. Image of a Western blot of Gfp mutants. A polyclonal antibody was used to probe for presence of Gfp. rGfp standards from *E. coli* were used to quantitate the amount of Gfp expressed. Degradation products are seen only in lanes with *E. coli* standards. Gfp expressed by CHO cells was the same size as the Gfp expressed by *E.coli*. Cells expressing CYC3 mutant expressed maximum amounts of protein.

of Gfp expressing cells to the amount of protein expressed per cell gave a measure of the relative brightness of mutant Gfps (Table 4A). According to this measure, mutants FM1 and FM2 can be considered to be the brightest Gfps, followed by the CYC3 mutant. The S65T mutation resulted in less than 2-fold increase in brightness relative to wildtype Gfp.

3.3. Detection of single gene copy expression

To determine whether single gene copy expression of Gfp was detectable, *S. cerevisiae* YPH399a cells were transformed with four yeast centromeric plasmids containing different Gfps (Fig. 1a). The CEN/ARS region restricts plasmid copy number to 1–2 per cell. Although the resulting plasmids were almost identical, there were some variations in the sequences immediately upstream of the structural gene (Fig. 1b). The transformed

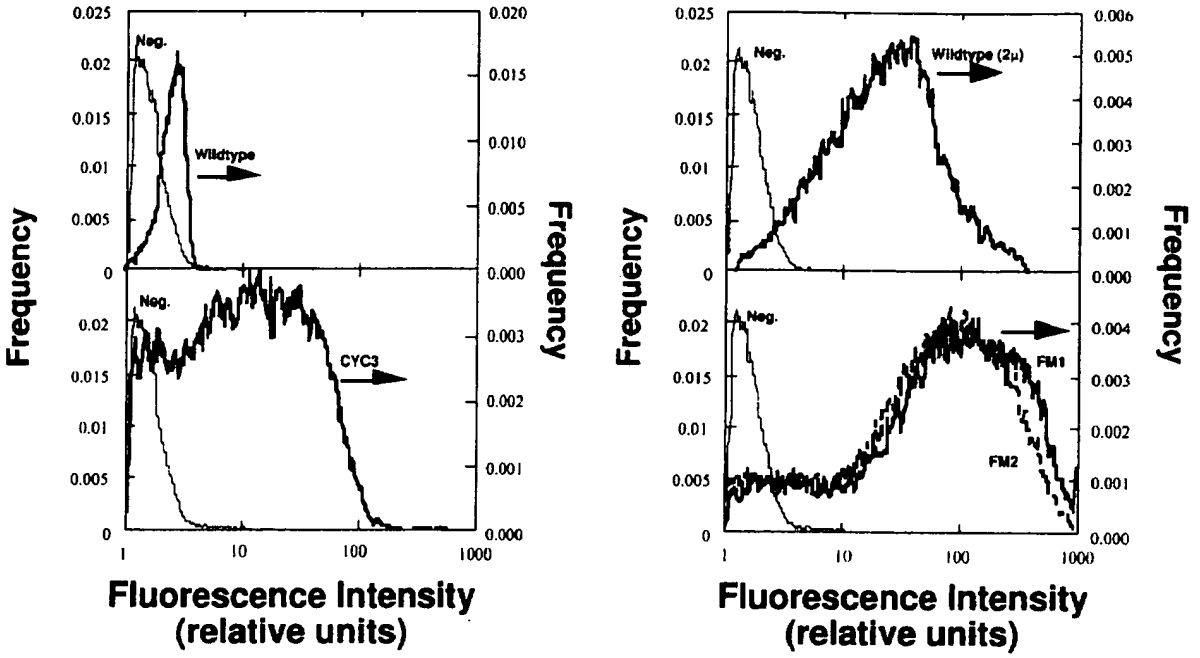

Fig. 6. Comparison of frequency distributions of various Gfps expressed in yeast cells. Wildtype Gfp was expressed from a 2 μ plasmid ('wildtype (2 μ)') and from a centromeric plasmid ('wildtype'). Gfp mutants were expressed from centromeric plasmids. Frequency distributions of the negative control ('Neg.', host strain with the parent centromeric plasmid) are shown on all panels and they correspond to the Y_1 axis (0–0.025). Frequency distributions of the wildtype and mutant Gfps correspond to the Y_2 axis. Distributions of mutants FM1 and FM2 were acquired at a lower gain setting of photomultiplier tubes than all other Gfps. A comparison of the mean fluorescence levels is provided in Table 4B.

yeast cultures expressed steady state levels of Gfp when grown on galactose (Fig. 6). Single copy expression of wildtype Gfp from the GAL1 promoter could not be clearly distinguished from background fluorescence levels, and this is contrary to the report of Niedenthal et al. (1996). Detectable fluorescence levels could be obtained only when the same construct was expressed from a multicopy (2 μ) plasmid. In contrast, mutant Gfps were clearly detectable even when expressed from a centromeric plasmid. In fact, mutants FM1 and FM2 offered impressive detection sensitivity as even 1–2 gene copies gave rise to very strong single cell fluorescence signals. A comparison of the relative brightness of mutant Gfps (Table 4B) indicates that mutants FM1 and FM2 show over 100-fold increase in fluorescence compared to wildtype Gfp. However, mutant CYC3 shows significantly less improvement over wildtype Gfp when expressed from a single copy vector. Attempts to detect expression of the S65T mutant in *S. cerevisiae* after direct cloning or cloning PCR-generated product into the same expression vector were unsuccesful. Although restriction analysis of these expression vectors confirmed the presence of the desired gene, it is as yet unclear whether errors in the gene, or expression vector, or other factors are responsible for this result.

3.4. Intracellular stability of green fluorescent protein mutants

In *S. cerevisiae* YPH399a cells growing exponentially on galactose, the GAL1 promoter was presumably fully induced, and cells reached a steady state level of Gfp expression. When glucose was added to the growth medium, transcription of Gfp mRNA from the GAL1 promoter was immediately inhibited due to glucose repression (Johnston et al., 1994). There was a perceptible lag of approximately 1 h after the addition of glucose to

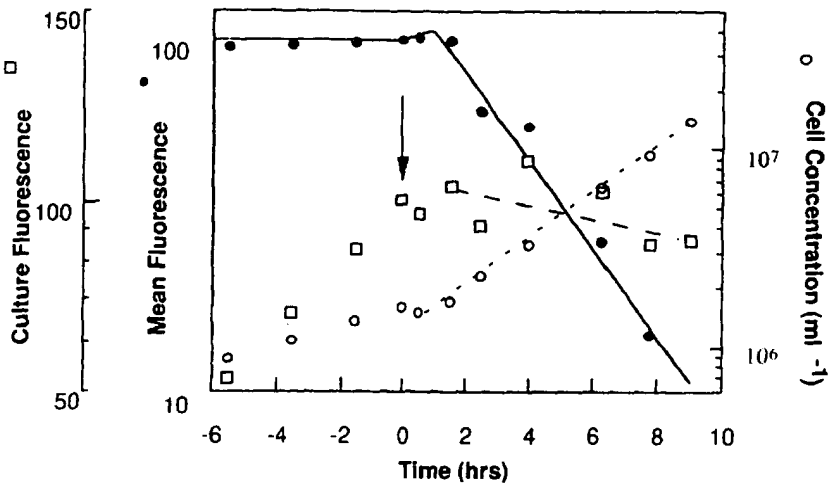

Fig. 7. Steady state Gfp expression was observed when cells grew exponentially on galactose. Glucose was added to the growth medium at time point '0' (arrow). The growing culture was repeatedly diluted to maintain cell concentrations less than 5×10^5 cells ml^{-1}, thus ensuring balanced growth. Subsequent to termination of transcription, there was no significant increase in the mean fluorescence of the culture (□, measured as the product of mean single-cell protein content and the cell density and normalized to 100 relative units at time point '0'). After the culture began growing exponentially on glucose, mean single-cell fluorescence decreased due to protein degradation (●) and dilution due to growth ( ). The rate of degradation was estimated by observing the rate of decrease of the mean fluorescence of the culture ( ), and by comparing observed decrease in intracellular fluorescence to simulated kinetics of Gfp expression ( ) based on a structured model developed previously (Subramanian and Srienc, 1996). Numerical values used in simulations were $m_0 = 10$ mRNA molecules per gene copy per cell (Iyer and Struhl, 1996), $S_n = 300$ protein molecules per mRNA transcript per hour (Petersen and McLaughlin, 1973), $S_f = 0.3456\ h^{-1}$ or $1.386\ h^{-1}$ (Heim et al., 1995), $\mu = 0.11\ h^{-1}$ or $0.269\ h^{-1}$ (experimental observation), $D_m 0 = 4.158\ h^{-1}$ or $0.462\ h^{-1}$ (Santiago et al., 1986). Symbol nomenclature is as defined in Subramanian and Srienc (1996). Stability of Gfp mRNA was found to have a negligible effect on the rate of protein degradation determined.

the growth medium prior to resumption of exponential growth, and mean Gfp content of cells increased slightly during this period. Presumably, cellular metabolism was shifting to growth on glucose during this time. Subsequent to this, the mean single cell fluorescence of the population decreased due to protein degradation and growth-associated dilution. The rate of decrease of fluorescence and the specific growth rate were measured and used to estimate the rate of intracellular Gfp degradation after assuming that there was no increase in number of fluorescent protein molecules after the initial lag period. This assumption was validated by plotting the total amount of fluorescence in the culture, which does not increase significantly after addition of repressor (Fig. 7). To account for the processes of continued translation and fluorophore formation after termination of transcription, a simple structured model developed previously (Subramanian and Srienc, 1996) was used to describe this transient behavior, and a similar conclusion was drawn by comparing experimental estimates with model simulations. Wildtype Gfp with an in vivo 1/2-life of approximately 7 h (≈ 3 doubling times) was the least stable Gfp (Table 4B). Improved solubility of all mutants was correlated with their increased stability (2–10-fold). The CYC3 mutant containing the V163A mutation was the most stable in *S. cerevisiae*, and is likely to be the most stable Gfp in CHO cells also, since transiently transfected cells produced highest amounts of CYC3 Gfp (Fig. 5).

4. Discussion

Mutants with stronger green fluorescence phenotype can be obtained by increasing the molar extinction coefficient, quantum yield, in vivo sta-

bility, or solubility, altering the excitation maxima to desired wavelengths, or by decreasing incorrect folding of nascent polypeptides into non-fluorescent molecules. Mutants FM1, FM2, and S65T have altered Ser-65 residues, which results in red-shifted excitation profiles and increased molar extinction coefficients. Mutants FM1, FM2, and CYC3 possess mutations that increase the solubility of the protein. Mutant CYC3 possesses the V163A mutation that reportedly suppresses sensitivity of the protein to folding correctly at elevated temperatures, although such sensitivity has not been observed in all systems (Subramanian and Srienc, 1996; this work). Indeed, in this work, we have shown that green fluorescence of wildtype Gfp expressed in *S. cerevisiae* is detectable when cells are grown at 30°C, and fluorescence is co-localized with the location of Gfp molecules identified by immunofluorescence staining.

Recently, Yang et al. (1996) have compared transiently expressed Gfp mutants S65T, FM1, and 'humanized' S65T ('EGFP', improved codon usage) in mammalian cells. However, they have not accounted for variations in intracellular protein content, which would almost certainly result from differences in codon bias. We have expressed Gfp mutants in CHO cells after transfection under identical conditions. Single cell green fluorescence was estimated using flow cytometry, and normalized for protein content by immunofluorescence staining, and Western blotting. The results indicate that mutants FM1 and FM2 appear brightest (6-fold) in CHO cells, and mutants S65T and CYC3 showed 2- and 4-fold increase in brightness compared to wildtype Gfp.

To assess the brightness of mutants expressed from a single gene copy relative to the wildtype, we have transformed *S. cerevisiae* cells with centromeric plasmids containing the genes under an inducible promoter. Although the resulting plasmids differ slightly in the sequence upstream of the translation start site, these changes are unlikely to produce significant changes in protein expression levels. Unlike in higher eukaryotes (Kozak, 1986), the untranslated upstream sequence in a transcript is dispensable in *S. cerevisiae*, and translation is not affected by the distance between the 5′ end of the transcript and the translation start site (Yun et al., 1996). A comparison of fluorescence levels of Gfps expressed from these plasmids is hence expected to yield meaningful results on their relative brightness. Upon induction, mutant Gfp expression was clearly detectable over background autofluorescence from single gene copies, while wildtype Gfp could not be clearly distinguished from non-expressing cells. Fluorescence of cells expressing mutant Gfps FM1 or FM2 was clearly detectable over autofluorescence even after ethanol fixation. This potentially allows the use of these mutants as reporter proteins for studying cell cycle kinetics without perturbation of DNA staining efficiency.

Among the advantages Gfp offers as a reporter protein is the ability to track in vivo expression of Gfp and Gfp-tagged proteins in living cells. A recent report demonstrates the capability for on-line monitoring of Gfp expression, which is of particular interest for biotechnology applications involving bioreactor monitoring, process design, and control (Randers-Eichhorn et al., 1997). However, in order to use Gfp as a reporter in observing transient cellular events, the kinetics of response subsequent to bi-directional changes in gene expression levels have to be ascertained. Mutants with improved detection sensitivity and fluorophore formation kinetics that allow faster identification of increase in gene expression levels have been described. However, effects of these mutations on protein stability which affects response to a decrease in expression levels have not been addressed. We have determined the intracellular stability of wildtype and mutant Gfps in *S. cerevisiae* by following decrease in fluorescence subsequent to transcription termination. The use of general transcription or translation inhibitors such as phenanthroline or cycloheximide was avoided since these antibiotics significantly perturb growth physiology (Santiago et al., 1986). Instead, we have determined rates of degradation under normal growth conditions by using a specific transcriptional block, resulting in a decrease of approximately three orders of magnitude in the rate of transcription within minutes after addition of repressor. In order to describe underlying kinetics of mRNA degradation, protein translation and fluorophore formation, simulations from a

previously developed structured model (Subramanian and Srienc, 1996) were compared to experimental data, and found to correspond very well. All Gfps were found to be very stable, thus undermining their use as cell cycle reporters. Interestingly, improved solubility of mutant Gfps was correlated with increased stability. In order for Gfp to be truly useful for studying transient phenomena, mutants have to be de-stabilized without compromising their improved fluorescent properties. The structured model developed previously is useful in estimating the maximum degree of destabilization for a given threshold of detection, and the model predicts that mutant FM1 can be detected with sufficient sensitivity over autofluorescence in *S. cerevisiae* with an engineered 1/2-life of approximately 20 min.

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